Phytochemistry of norditerpenoid alkaloids

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Abstract

Norditerpenoid alkaloids have important biological activities. Many of them are potent ligands and therefore promising leads for novel selective antagonists and/or agonists of sub-types of nicotinic acetylcholine receptors (nAChR) and voltage-gated sodium channels. *Aconitum*, *Consolida*, and *Delphinium* are important sources of norditerpenoid alkaloids. This thesis is focussed on the chemistry of norditerpenoid alkaloids from these three genera, starting with a review of those recently isolated from *Aconitum*, *Consolida*, and *Delphinium* with brief aspects of taxonomy, biological activities, and modes of action. Selected aspects of the uses in traditional medicine of *Delphinium* and *Aconitum* are presented, including data on both toxicity and detoxification. In this thesis, chemical constituents of the seeds of Delphinium cultivar Pacific Giant and the seeds of *Aconitum lycoctonum* were investigated.

To extract the crude alkaloidal material, Soxhlet extraction was used for Delphinium cv Pacific Giant seeds and room temperature extraction for *A. lycoctonum* seeds. The crude extracts were purified by repeated column chromatography (over silica and alumina gels), yielding five known norditerpenoid alkaloids from Delphinium cv Pacific Giant (delavaines A and B, delpheline, methyllycaconitine (MLA), and pacinine) and two from *A. lycoctonum* (lycaconitine and *N*-succinylanthranoyl lycoctonine) which were analysed by detailed spectroscopic methods. X-Ray crystallographic analysis of aconitine, mesaconitine, lycoctonine, and delpheline was also studied. Three compounds were obtained from semi-synthesis starting with MLA: lycoctonine by basic hydrolysis, inuline by acidic hydrolysis and by esterification of lycoctonine, and elatine by methylenedioxy acetal formation. These known alkaloids were structurally elucidated by a variety of spectroscopic techniques.

MLA is a selective competitive antagonist at α7 sub-type nAChR. From collaborative studies, the results of biological activity for methyl esters delavaines A and B (a 3:2 mixture), delpheline, and pacinine (the B-ring C6-ketone of delpheline) are reported in this thesis. Their biological activities were determined in competitive α-bungarotoxin binding assays for α7 nAChR in rat brain membranes. Delavaines A and B were potent ligands (IC_{50} = 50 nM, cf MLA IC_{50} = ~1-2 nM), whereas delpheline and pacinine displayed only modest activity at α7 nAChR (IC_{50} = ~1 μM).

After studying *N*-ethylpiperidine as a model alkaloid, the pK_a (a key physico-chemical parameter) of MLA was measured by ^{1}H NMR as 7.15. A brief comparison with pK_a data reported in the literature is made across closely related norditerpenoid alkaloids.

Acknowledgements

My greatest appreciation goes to my supervisors Dr. Ian S. Blagbrough and Dr. Michael G. Rowan for their excellent supervision, good advice, and support without which this work would not have been possible. They devoted their best to helping me to learn techniques that I have never learnt before. I have had a good experience working with them.

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Finally, but not least, I would like to thank my family for their support and for encouraging me through these studies.

Abbreviations

°C	degrees Celsius
Å	angstrom
Ac	acetyl
aq	aqueous
As	anisoyl
^{i}Bu	isobutyryl
Bz	benzoyl
CI	chemical ionisation
Cn	cinnamoyl
COSY	correlated spectroscopy
cv	cultivar
2D	two dimensional
DCM	dichloromethane
DEPT	distortionless enhancement by polarization transfer
EI	electron impact
ESI	electrospray ionisation
Et	ethyl
FAB	fast atom bombardment
HMBC	heteronuclear multiple-bond correlation
HMQC	heteronuclear multiple-quantum correlation
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
Hz	hertz
IR	infrared
J	coupling constant
LC-MS	liquid chromatography-mass spectrometry
LC-MS-MS	liquid chromatography-tandem mass spectrometry
M	molar
Me	methyl
MH^{+}	protonated molecular ion
mp	melting point
MS	mass spectrometry
MW	relative molecular weight
m/z	mass over charge

mAChR	muscarinic acetylcholine receptors
nAChR	nicotinic acetylcholine receptors
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
ppm	parts per million
^{i}Pr	isopropyl
RPC	reversed phase chromatography
rt	room temperature
TLC	thin layer chromatography
UV	ultraviolet
v/v	volume by volume
Vr	veratroyl

Contents

1. Introduction

1.1. Definitions of the norditerpenoid alkaloid class

The name alkaloid is derived from the word alkali. However, the degree of basicity varies greatly, depending on the structure of the alkaloid molecule and the presence and position of other functional groups. According to Pelletier[1], an alkaloid is a cyclic organic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms. Although most alkaloids are cyclic compounds displaying a wide variety of important biological activities, there are also biologically active linear alkaloids such as those in the polyamine class: spermidine and spermine.

Like other natural products, alkaloids may be classified by their common molecular features, based on the metabolic pathway used to construct the molecule. However, prior to the development of an extensive understanding of the biosynthesis of alkaloids, they were grouped under the names of known compounds, even some non-nitrogenous ones or by the plants or animals they were isolated from. Thus, alkaloids are commonly classified into the following groups: pyridine, pyrrolidine, tropane, quinoline, isoquinoline (opium alkaloids), phenethylamine, indole, purine, and terpenoid, together with vinca alkaloids. The names of these groupings are terms which often cut across biosynthetic pathways. Following from the suggestion by Hegnauer[2], true alkaloids are those in which the carbon skeleton derived from a precursor amino acid and formed into the heterocyclic system. In pseudo-alkaloids the carbon skeleton is not derived from an amino acid. Pseudo- seems etymologically an unsuitable prefix, because these compounds are considered to be genuine alkaloids by the above definition. These compounds are better described as crypto-alkaloids.

Diterpenoid alkaloids are thus crypto-alkaloids in which the carbon skeleton is derived from a diterpene unit (C_{20}), from geranylgeranyl pyrophosphate, through copalyl pyrophosphate, to *ent*-kaurene and then subsequent oxidation steps around the carbon skeleton. 2-Amino-ethanol is often the biosynthetic precursor for the *N*-ethyl moiety e.g. for atisine-type diterpene alkaloids on the pathway to aconitine-type norditerpenoid alkaloids. The latter are those in which the structural fragment is derived from a C_{20} terpenoid having lost one carbon atom during the biosynthesis. Norditerpenoid alkaloids of *Aconitum*, *Delphinium*, and *Consolida* are C_{19} hexacyclic alkaloids with many alkoxyl and/or hydroxyl and/or acyl groups. Pelletier divided norditerpenoid alkaloids into four types: aconitine type possessing the type I skeleton (Figure 1.1) with the absence of oxygenation at C-7, lycoctonine type

possessing the type I skeleton with the presence of oxygenation at C-7, pyrodelphinine type also possessing the type I skeleton with a double bond between C-8 and C-15, and heteratisine type possessing the type II skeleton (Figure 1.2) in which a lactone moiety is present in ring C.[3]

According to the divisions of Katz and Hanuman,[4] norditerpenoid alkaloids are divided into six types which possess the same skeleton (type I), and oxygenation at C-1, C-6 (except isotalatizidine type), C-8, C-14, C-16 and C-18: aconitine type with additional hydroxyl groups at C-3, C-13 and C-15, pseudaconitine type with additional hydroxyl groups at C-13 and C-15 only, bikhaconitine type with additional hydroxyl group at C-13 only, neoline type without the additional hydroxyl group, isotalatizidine type without the oxygenation at C-6, and lycoctonine type with the additional oxygenation at C-7 and, in general, oxygen substitution at C-6 on the β-face. The aconitine, pseudaconitine, bikhaconitine, and neoline types have oxygen substitution at C-6 on the α-face. However, neither of these chemically defined classification schemes entirely correlates with either pharmacological activity or plant taxonomic position.

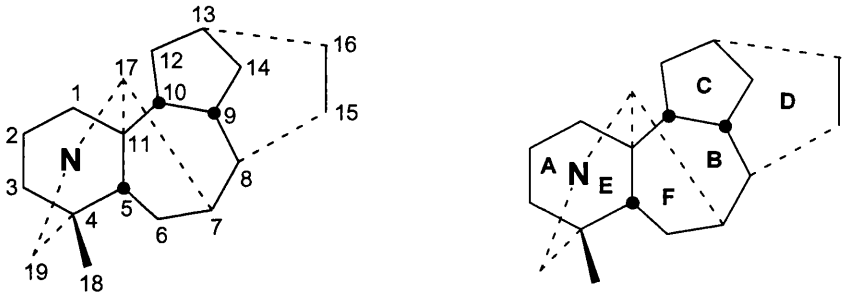


Figure 1.1 Type I skeleton of norditerpenoid alkaloids a) numbering system, b) ring names

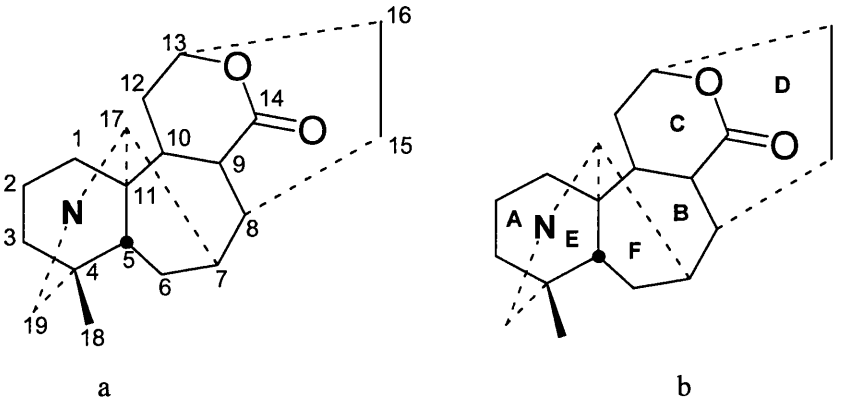


Figure 1.2 Type II skeleton of norditerpenoid alkaloids a) numbering system, b) ring names

1.2. Taxonomy of plants examined

1.2.1. *Aconitum lycoctonum*

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Ranunculales
Family:	Ranunculaceae
Subfamily:	Ranunculoideae
Tribus:	Delphinieae
Genus:	*Aconitum*
Species:	*Aconitum lycoctonum*

The common name of this plant is wolfsbane. In epithetical, lycoctonum means wolf slaying. Other synonyms that have been used by various authorities are *A. lycoctonum* subsp. *vulparia*, *A. septentrionale* and *A. vulparia*. This herbaceous perennial plant is chiefly native of the mountainous parts of the northern hemisphere from Europe to West Asia, growing in damp soils on mountain meadows and in heavy clay soils in open woodland. The general characteristic of *Aconitum* was shown in the following. [5] Their dark green leaves, lacking stipules, are palmate or deeply palmately lobed with 5–7 segments. Each segment again is 3-lobed with coarse sharp teeth.

The leaves have a spiral or alternate arrangement.

The lower leaves have long petioles.

The tall, erect stem is crowned by racemes of large and eye-catching blue, purple, white, yellow or pink zygomorphic flowers with numerous stamens.

They are distinguishable by having one of the five petaloid sepals (the posterior one), called the galea, in the form of a cylindrical helmet; hence the English name monkshood.

There are 2–10 petals, in the form of nectaries.

The two upper petals are large, placed under the hood of the calyx and are supported on long stalks.

They have a hollow spur at their apex, containing the nectar.

The other petals are small or completely lacking.

The 3–5 carpels are partially fused at the base.

The fruit is a follicle.

1.2.2. Delphinium cv. Pacific Giant

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Ranunculales
Family:	Ranunculaceae
Subfamily:	Ranunculoideae
Tribus:	Delphinieae
Genus:	*Delphinium*

Delphinium is a genus of about 250 species of annual, biennial or perennial flowering plants in the buttercup family Ranunculaceae, native throughout the Northern Hemisphere and also on the high mountains of tropical Africa. The common name, shared with the closely related genus *Consolida*, is larkspur. *Consolida* is sometimes included in the genus *Delphinium* in the trade but it is considered a separate genus botanically. *Consolida* differs from *Delphinium* in having leaves palmately highly dissected into narrow, flilform segments, and flowers with two upper petals united into one, and two lower petals absent. Many species are cultivated. Other species or hybrids are *D. X belladonna* and *D. grandiflorum*. *D. x elatum* 'Pacific Giant' series is an old cultivar developed in the 1930s in California. The general characteristics of *Aconitum* were shown in the following:[6]

these cultivars are available with solid and bicolor flowers, as well as a mix, varying between purple, blue, white or mixed;

the leaves of *Delphinium* are deeply lobed with 3-7 toothed, pointed lobes;

the main flowering stem is erect, and varies greatly in size between the species, from 10 cm in some alpine species, up to 2 m tall in the larger meadowland species; it is topped by many flowers;

the spurred, zygomorphic flowers in spikelike racemes have petaloid calyx of five petals approximately 1-2.5 cm long, one of which is elongated into a spur approximately 1-2 cm long;

the corolla is small and forms the central "bee" of four petals in two unequal pairs, upper pair extending into spurs that project into sepal spur and lower pair sometimes bearded; the flowers have many stamens; the seeds are small and shiny black;

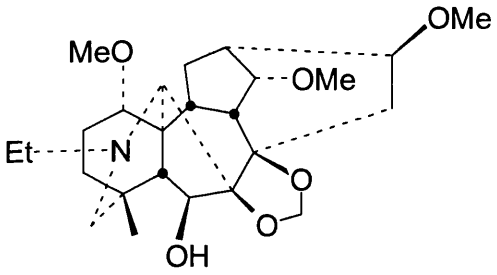
the plants flower from late spring to late summer, and are pollinated by butterflies and bees.

1.3. Occurrence of norditerpenoid alkaloids

1.3.1. Overview of the norditerpenoid alkaloid literature

As the reporting of naturally occurring complex norditerpenoid alkaloids is both widespread and ever expanding, this review has been focussed upon selected more recent reports (essentially 1999-2006 following on from the last major reviews)[7-11] and on those alkaloids with either established biological activity or those with more complex substitution patterns. It is divided into the three genera: *Aconitum, Delphinium*, and *Consolida.*

Aconitum and *Delphinium* have long been recognized as a source of both diterpenoid and norditerpenoid alkaloids. More than 300 norditerpenoid alkaloids have been isolated from many plants in the genera *Aconitum, Delphinium*, and *Consolida*, including many recently described. Alkaloids reported prior to 1999 have been extensively reviewed in various papers and book chapters by Pelletier,[7-11] Yunusov,[12, 13] and Atta-ur-Raman.[14-16] The alkaloids isolated from *D. elatum* L., cultivated to Delphinium cv Pacific Giant, are: delpheline **1**[17], delelatine **2**[18], elatine **3**[19], methyllycaconitine (MLA) **4**[17], and deltaline **5**[19].



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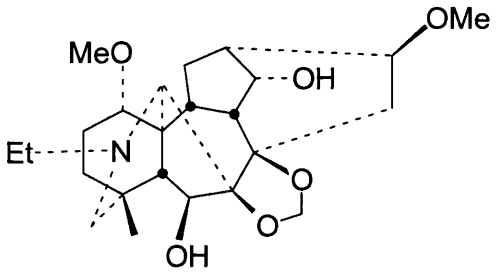

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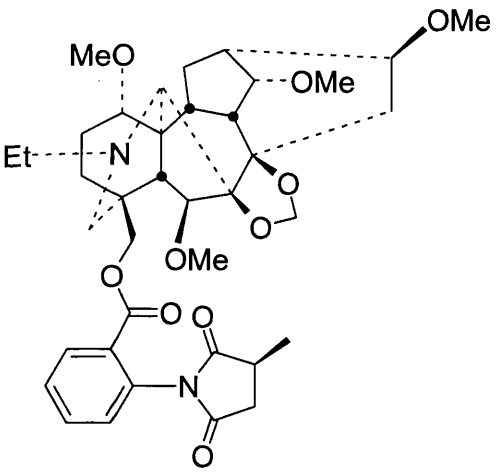

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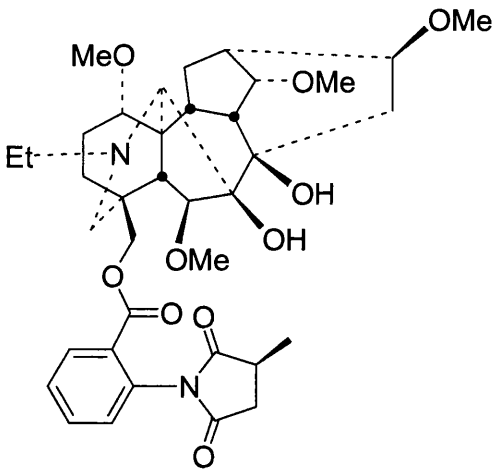

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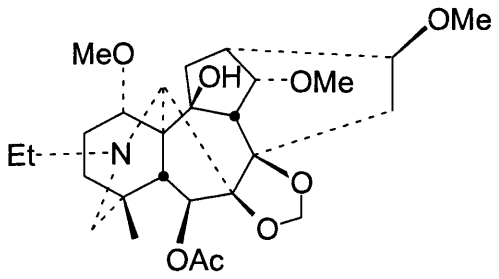
A similar series of compounds has been reported from the so-called tall larkspurs of North America, *D. barbeyi* Huth, *D. glaucum* S. Wats, and *D. occidentale* S. Wats yielding norditerpenoid alkaloids: delpheline **1**[20], deltaline **5**[20], lycoctonine **6**[20, 21], and anthranoyl lycoctonine (inuline) **7**[21] from *D. barbeyi* Huth and *D. glaucum* S. Wats, and delpheline **1**[22] and deltaline **5**[22] from *D. occidentale* S. Wats. In early studies (in 1884)[23] on *A. lycoctonum*, Dragendorff and Spohn reported lycoctonine **6**[23] and lycaconitine **8**[23]. This was followed by a 1913 paper[24] from Schulze and Bierling on these alkaloids which are still of interest today.



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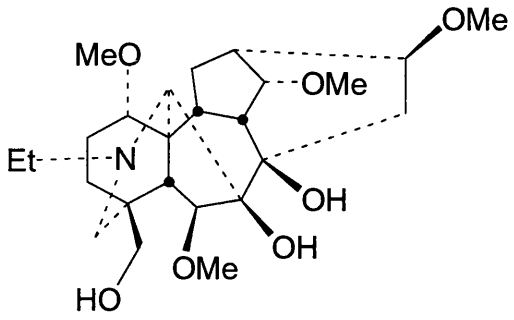

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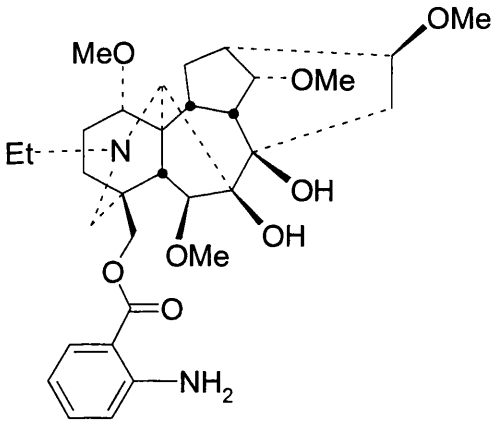

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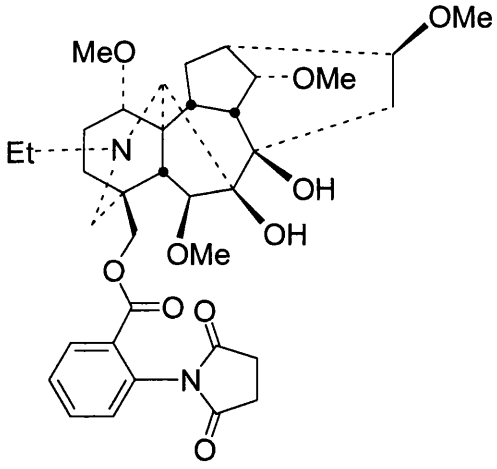

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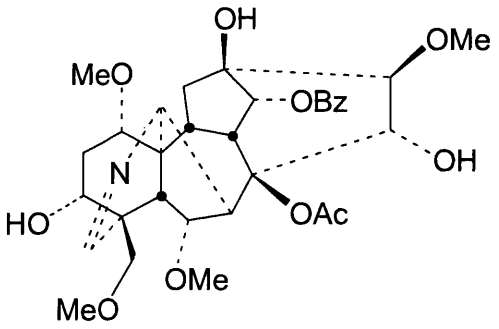
1.3.2. Norditerpenoid alkaloids from *Aconitum*

From the genus *Aconitum*, Pelletier and co-workers reported a new norditerpenoid alkaloid, imine merckonine **9** from a commercial source "Aconitine Potent Merck", isolated from *A. napellus* L., Lot. No. 30169.[25] From the seeds of *A. barbatum* Pers., Pelletier and co-workers reported four known diterpenoid alkaloids and one known norditerpenoid alkaloid, delcosine **10**.[26] Furthermore, they isolated four known norditerpenoid (altaconitine **11**, aconitine **12**, senbusine A **13** and neoline **14**) and three known diterpenoid alkaloids from the aerial parts of *A. volubile* Pall.[26]



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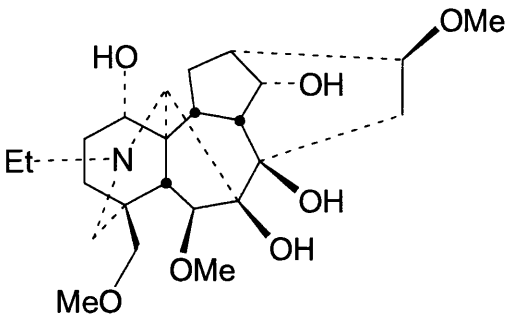

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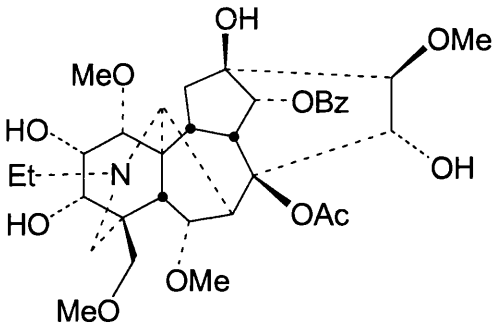

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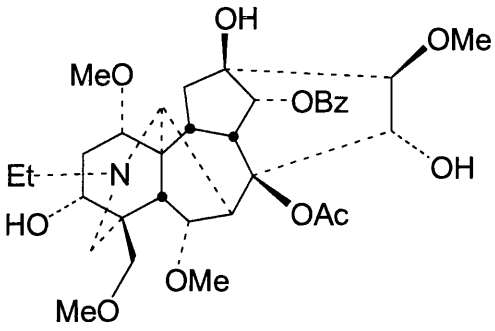

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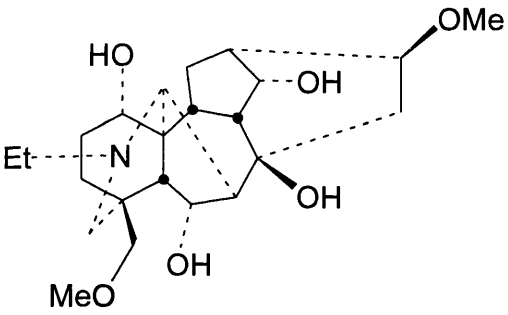

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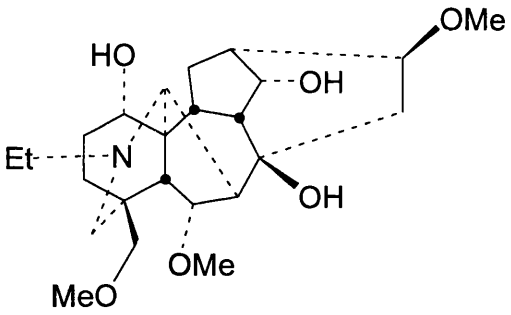

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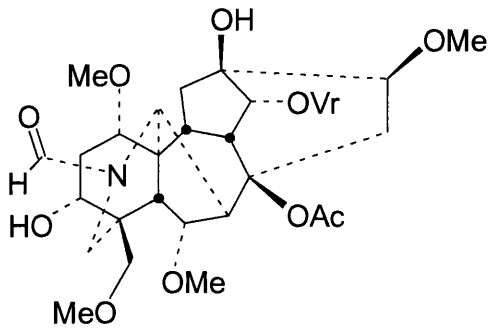
Attar-ur-Rahman and co-workers isolated two new norditerpenoid alkaloids, faleoconitine **15** and 3′-methoxyacoforestinine **16** along with three known compounds from the roots of *A. falconeri*.[27] The structure of faleoconitine **15** shows the formyl group attached to the nitrogen atom instead of alkyl group like most norditerpenoid alkaloids and is presumably not basic. Choudhary and co-workers reported two new norditerpenoid alkaloids, acofamine A **17** and acofamine B **18**, were isolated from the aerial parts of *A. karakolicum* Rapaics.[28] From the roots of the same species collected in Kirghizstan, Robert and co-workers reported a new alkaloid, 8-*O*-azeloyl-14-benzoylaconine **19**.[29] Long chain fatty acyl esters at C-8 have been reported previously, but the incorporation of a long chain dicarboxylic acid allows the formation of a zwitterion with the nitrogen atom.



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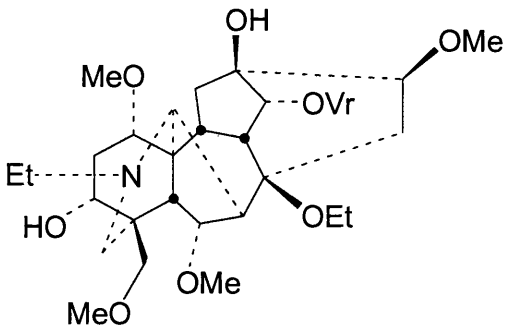

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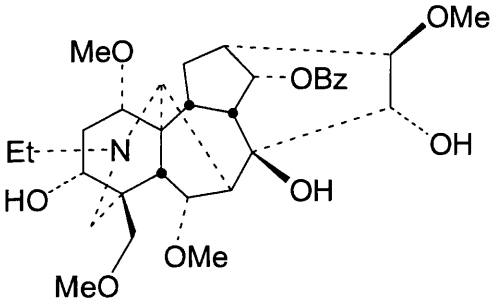

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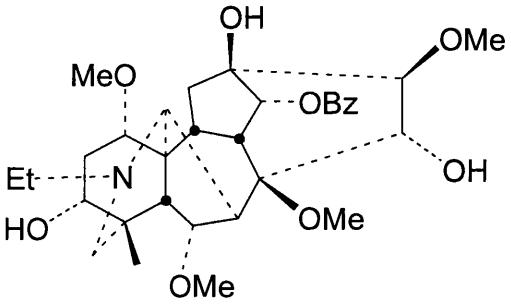

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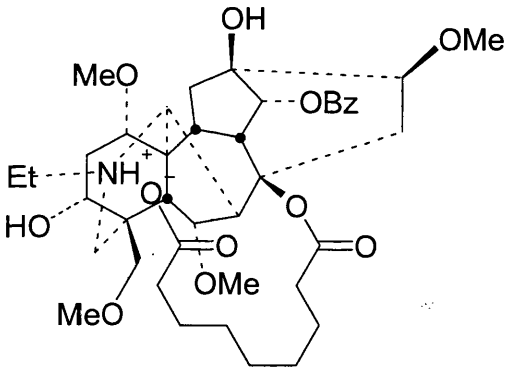

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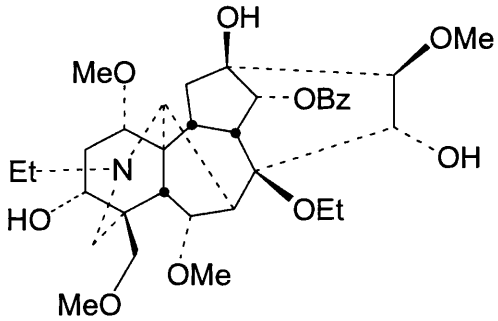

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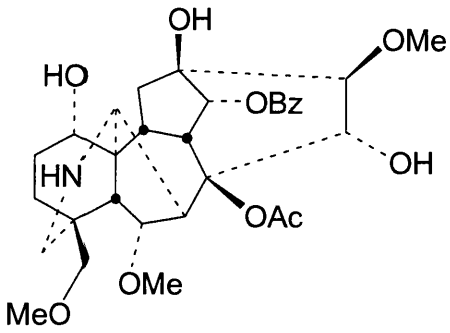

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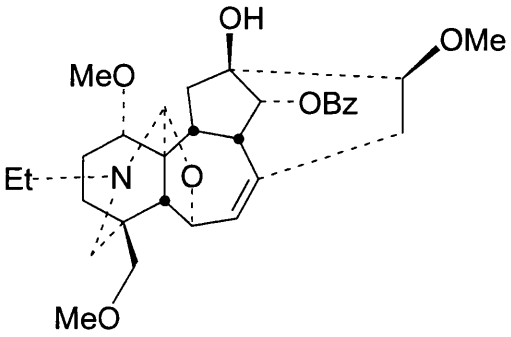

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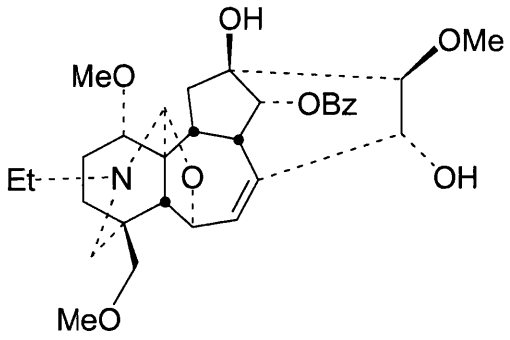

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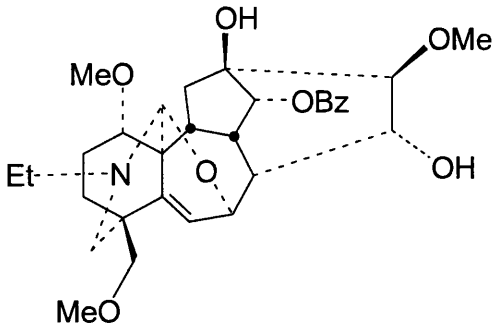
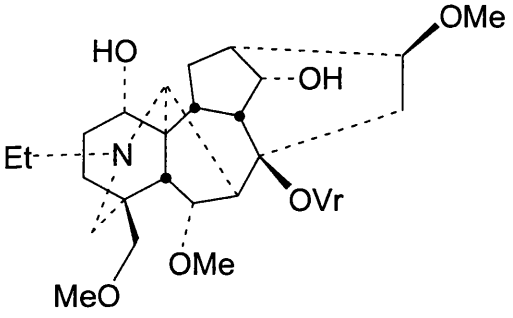


24

Gao and co-workers reported two new norditerpenoid alkaloids, spicatine A **20** and spicatine B or 10-dehydroxyflavaconitine **21**, as well as eleven known norditerpenoid alkaloids from the root of *A. spicatum* Stapf.[30] Ding and co-workers reported one new norditerpenoid alkaloids, 13-hydroxyfranchetine **22**, and one known alkaloid, 10-dehydroxyflavaconitine **21**, from the roots of *A. nagarum*.[31] Che and co-workers isolated beiwudine **23** and three known compounds from the roots of *A. kusnezoffii*.[32] In the roots of same species, Yunusov and co-workers reported a new alkaloid acsonine **24** and a known compound mesaconitine **37**.[33] 13-Hydroxyfranchetine **22** differs from beiwudine **23** by absence of hydroxyl group at C-15. The structures of 13-hydroxyfranchetine **22** and beiwudine **23** are atypical: no carbon-carbon bond at C-7 and C-17, but carbon-oxygen at C-17 and *O* attached to C-6 forming a tetrahydrofuran ring instead of a five-membered ring, ring F, and a double bond at C-7 and C-8. The structure of acsonine **24** is also atypical: no carbon-carbon bond at C-7 and C-17, but carbon-oxygen at C-17 and *O* attached to C-7 forming a six-membered ring instead of a five-membered ring, ring F and a double bond at C-5 and C-6. Hohmann and co-workers reported acotoxinine **25** isolated from the roots of *A. toxicum* Rchb., together with two known norditerpenoid alkaloids and two known diterpenoid alkaloids.[34] It is unusual in having an aromatic acyl group at C-8 rather that C-14. Pelletier and co-workers reported a new diterpenoid alkaloid and three known norditerpenoid alkaloids, lappaconitine **26**, lycoctonine **6**, and gigactonine **27** from the roots of *A. nasutum* Fisch. ex Reichb.[35]



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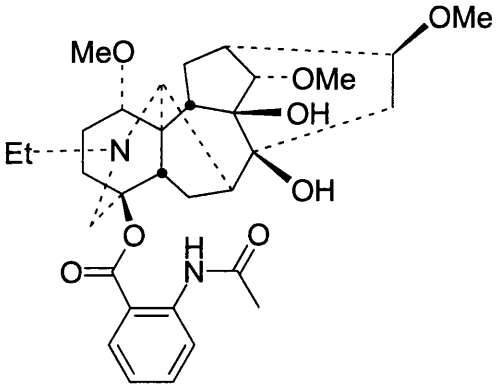
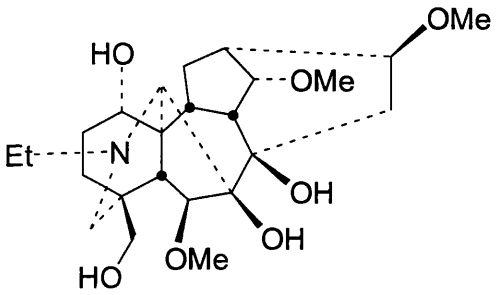


26

Wang and co-workers reported four new norditerpenoid alkaloids, geniculatines A **28**, B **29**, C **30**, and D **31**, from the roots of *A. geniculatum* Fletcher.[36] From the roots of *A. sinomontanum*, Wang and co-workers isolated five new norditerpenoid alkaloids, sinomontanitine A **32** and B **33**, and sinomontanine A **34**, B **35**, and C **36**, along with two known compounds.[37] Like lappaconitine **26**, sinomontanine A **34** and B **35** might be considered to be a bisnorditerpenoid alkaloid. Sinomontanitine A **32** and B **33** have the usual acyl group, succinimidobenzoyl, at the unusual position, C-8. In general, this acyl group attaches the position at C-18 and has strongly effect to toxicity.



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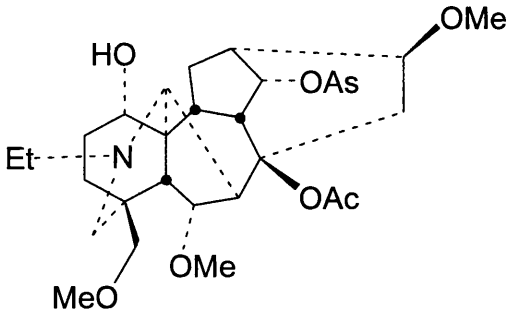

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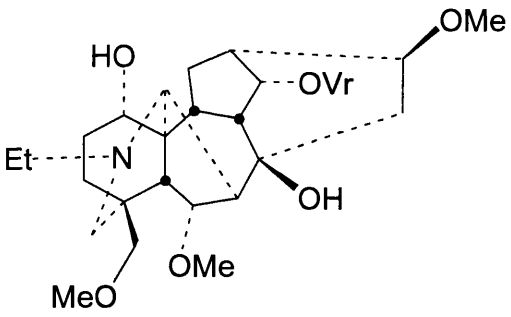

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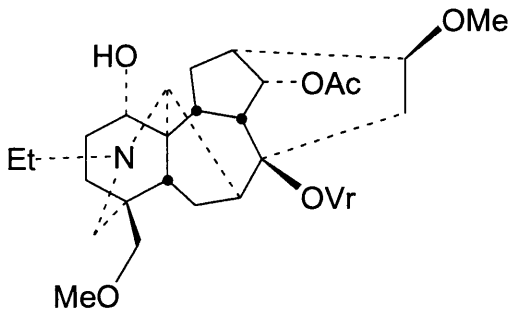

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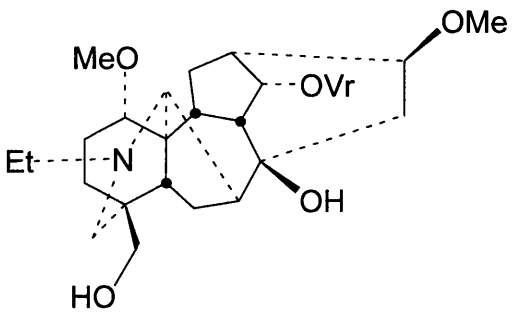

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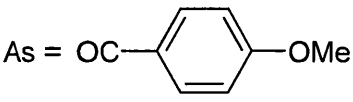
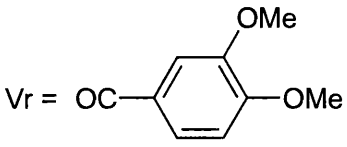
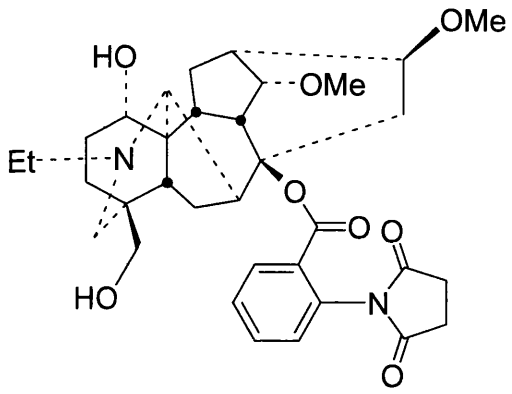


As = anisoyl



Vr = veratroyl



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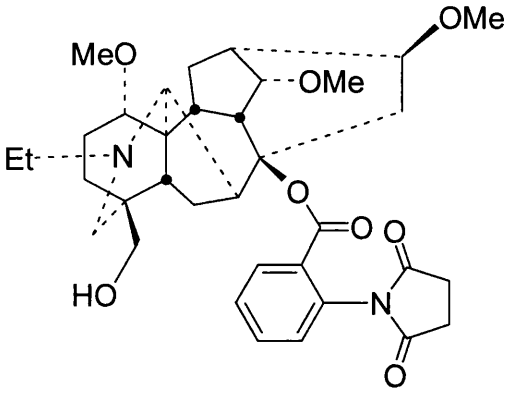
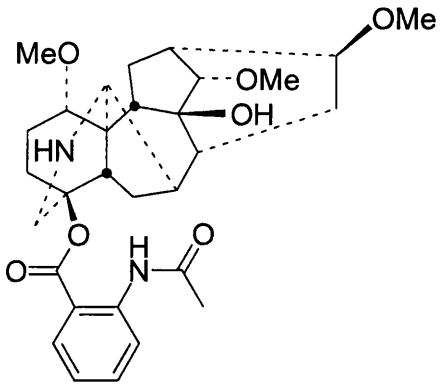


33

Kang and co-workers isolated seven norditerpenoid and one diterpenoid alkaloids from *A. jaluense*. The norditerpenoid alkaloids were identified as neoline **14**, mesaconitine **37**, hypaconitine **38**, lipomesaconitine, lipohypaconitine, 15α-hydroxyneoline, and hokbusine A **39**.[38] The four latter have not been found from this plant before. From the processed tubers of *A. carmichaeli*, Kang and co-workers reported four new (14-*O*-cinnamoylneoline **40**, 14-*O*-anisoylneoline **41**, 14-*O*-veratroylneoline **42** and 14-*O*-anisoylbikhaconine **43**) and five known norditerpenoid alkaloids.[39]



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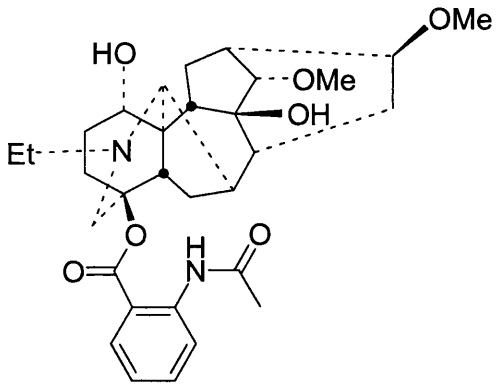

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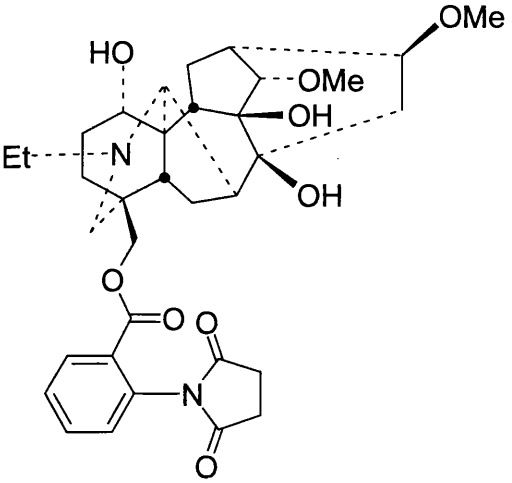

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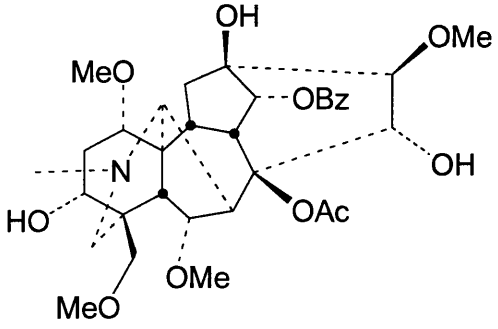

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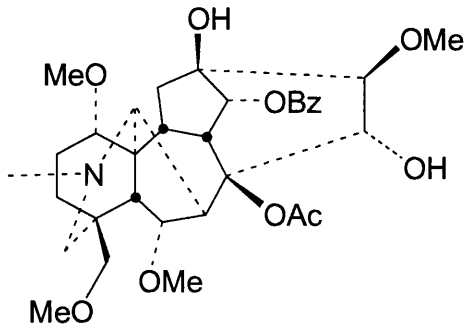

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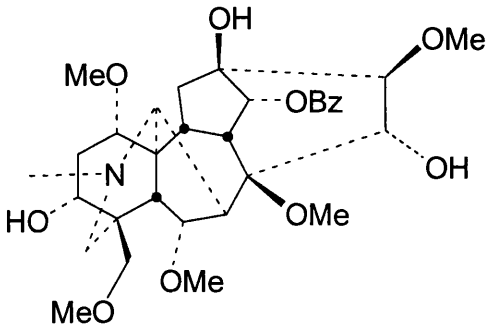

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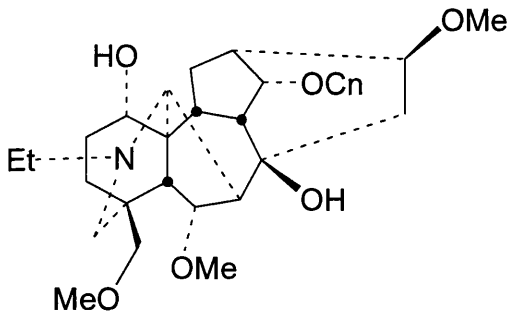

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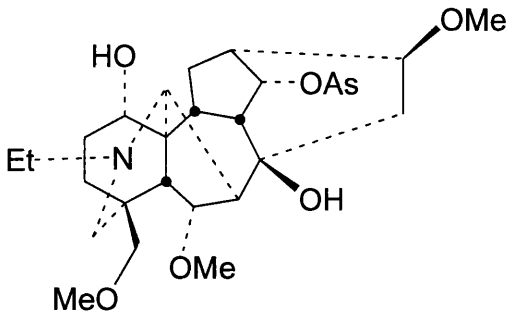

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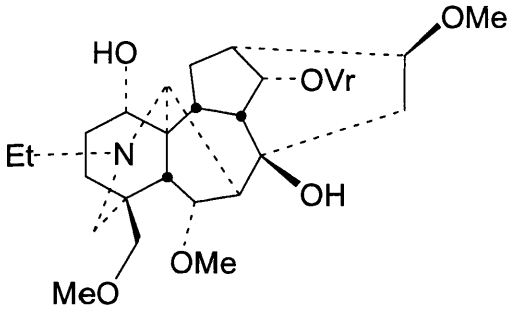

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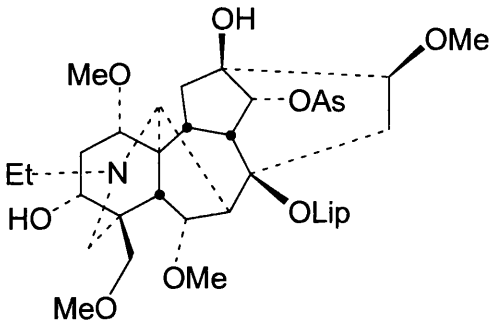
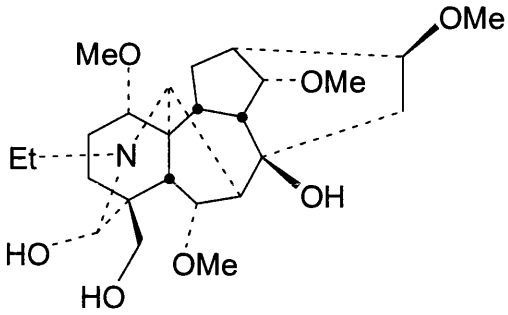


Lip = linoleoyl, palmitoyl, stearoyl, oleoyl

43

Mericli and co-workers reported two new norditerpenoid alkaloids, one was a non-arylated aconitine type named aconitilearine **44**, extremely unusual in possessing hydroxylation at both C-18 and C-19, and *N*-deethylmethyllycaconitine **45**, along with the eight known norditerpenoid alkaloids from the aerial parts of *A. cochleare* Woroschin.[40] It is extremely unusual to find the MLA acyl group in an *Aconitum* species. Khetwal and Pande isolated a new norditerpenoid alkaloid from the roots of high altitude Himalayan herb *A. balfourii*, namely 9-hydroxysenbushine A **46**.[41]



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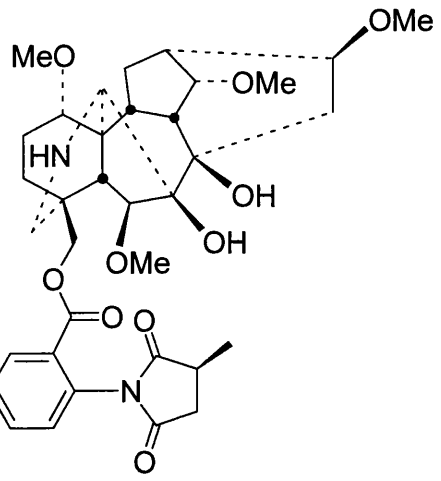

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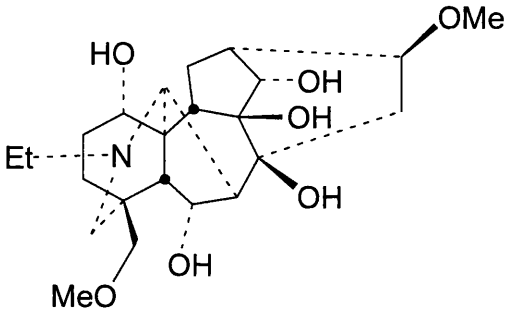

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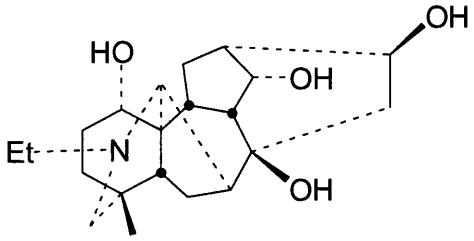
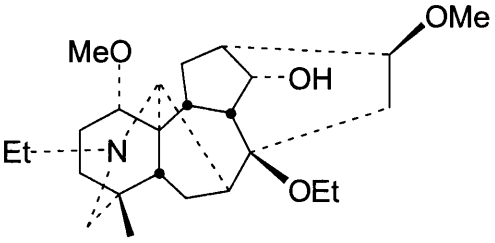


47

Herz and co-workers investigated the aerial parts of *A. variegatum* L. from the Pyrenees, furnishing four new norditerpenoid alkaloids: 16β-hydroxycardiopetaline **47**, 8-ethoxysachaconitine **48**, 14-acetylgenicunine B **49** and the naturally occurring imine *N*-deethyl-*N*,19-didehydrosachaconitine **50**, five new diterpenoid alkaloids and thirteen known alkaloids.[42] *N*-Deethyl-*N*,19-didehydrosachaconitine **50** is unusual as it is an imine.



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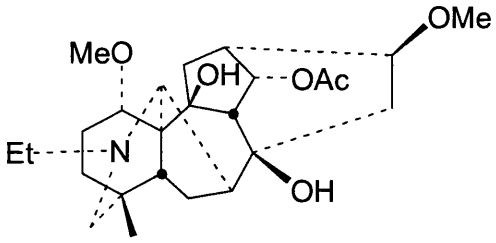

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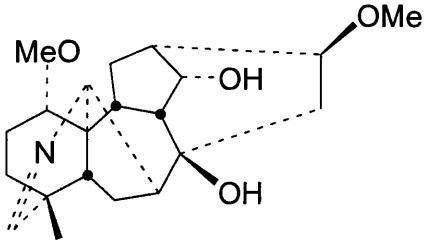

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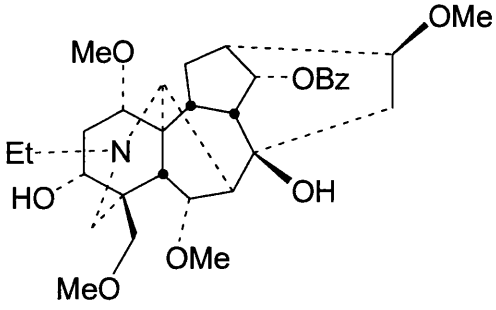

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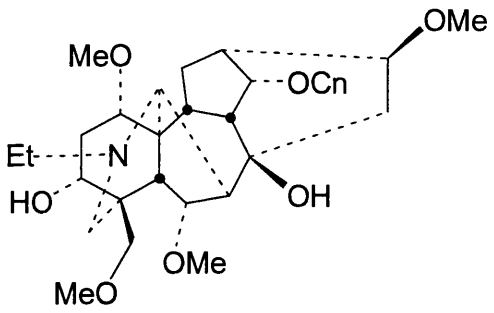

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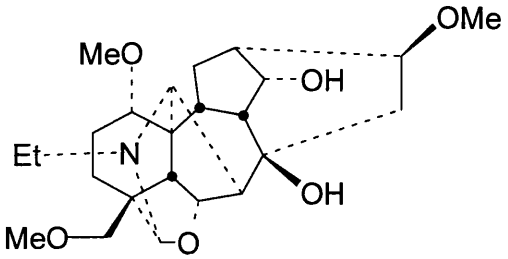
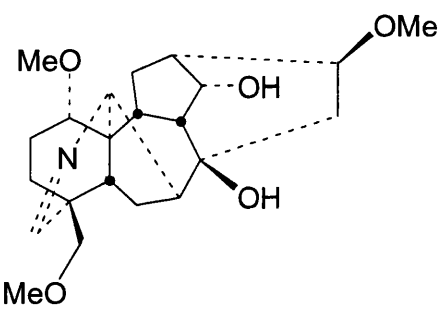


53

Wang and co-workers reported five new norditerpenoid alkaloids, 13-deoxyludaconitine **51**, 8-deacetylsungpaconitne **52**, pengshenine A **53**, pengshenine B **54**, and hemsleyatine **55**, from the roots of *A. hemsleyanum* Pritz *var. pengzhouense*.[43-45] Like merckonine **9** and *N*-deethyl-*N*,19-didehydrosachaconitine **50**, pengshenine B **54** is an imine. The structure of pengshenine A **53** shows an ether bond between C-6 and C-19, making a complex structure. Unlike other norditerpenoid alkaloids, hemsleyatine **55** has an amino group attached to C-8 instead of oxygenation at C-8. However, as 10% ammonium hydroxide was used during the isolation, the primary amino group could be an artefact.



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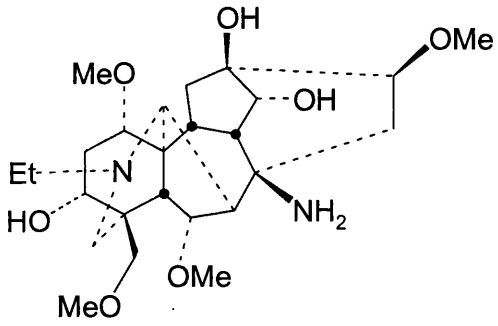

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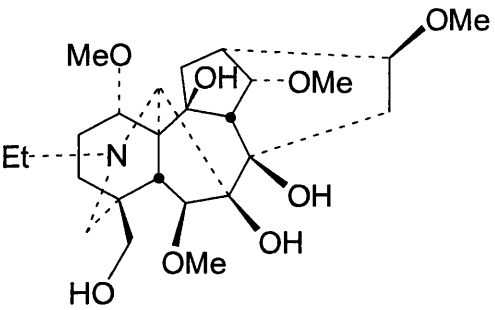

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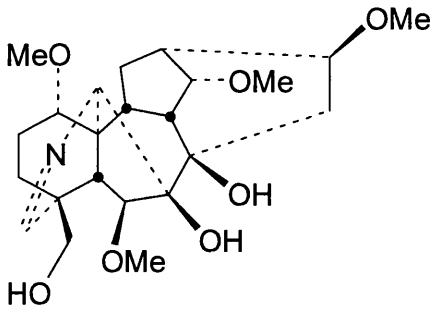
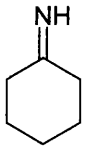


57

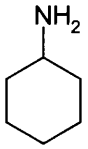
Shaheen and co-workers isolated a new norditerpenoid alkaloid of the lycoctonine type with oxygenation at C-7 from the aerial parts of *A. laeve* Royle, namely swatinine **56**, and four known compounds.[46] Hohmann and co-workers isolated a new norditerpenoid alkaloid,

acovulparine **57**, and two known compounds lycoctonine **6** and delcosine **10** from the whole plants of *A. vulparia* Reichenb that some botanical authorities considered to be synonymous with *A. lycoctonum*.[47] Acovulparine **57**, with its carbon-nitrogen double bond, is also an imine (cf **9**, **50**, and **54**) which will have an impact on the basicity of these alkaloids.

Imines can arise biosynthetically by oxidation (e.g. cytochrome P450 catalysed) of amines followed by dehydration to make the C=N, or by dehydration of a hemiaminal, itself derived by nucleophilic attack of an amine upon an aldehyde functional group. Whilst, in organic and biological chemistry, imines are generally viewed as somewhat unstable, it is clear that they are sufficiently stable plant natural products to undergo extraction procedures including various pH steps and dissolving in alcohols. The experimental pK_a values along the series: piperidine, *N*-methyl, *N*-ethyl- are: 11.02, 10.38, 10.45 (see section 3.6), the cyclic imine piperideine has a calculated (ACD package) pK_a of 7.46. The basicity of cyclohexanimine (pK_a = 9.15)[48] (Figure 1.3) is less than that of cyclohexylamine (pK_a = 10.64) due to the 33% s-character of the hybrid sp^2 orbital of the nitrogen of cyclohexanimine being higher than 25% s-character in the sp^3 orbital of the nitrogen atom of cyclohexylamine. Thus, the lone pair of electrons stays close to the *N*-atom nucleus and so is less available as a base. In general, this explains why the basicity of amines is higher than that of the corresponding imines. Their basicity might affect their biological activities (see section 1.4.4).

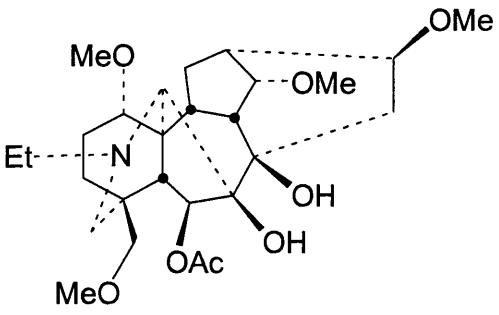


cyclohexanimine (pK_a = 9.15)

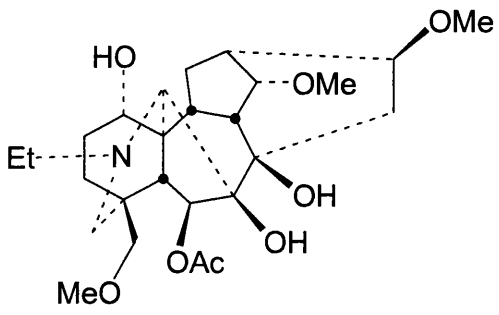


cyclohexylamine (pK_a = 10.64)

Figure 1.3 pK_a for an imine and its related amine



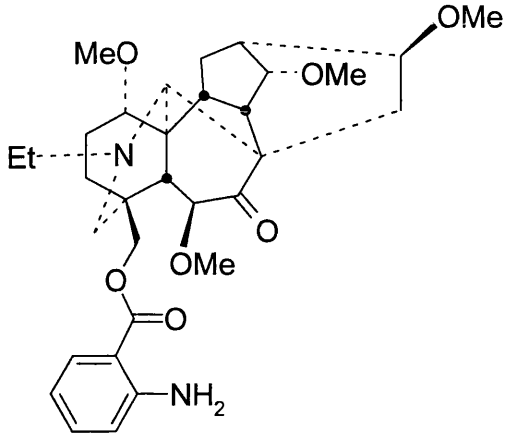
58



59

Chen and Katz reported two new norditerpenoid alkaloids, 6-*O*-acetyldemethylenedelcorine **58** and 6-*O*-acetyl-14-*O*-methyldelphinifoline **59**, and three known compounds, 14-*O*-methyldelphinifoline, gigactonine, and lycoctonine, from the flowers of *A. lycoctonum*.[49]

Usmanova and co-workers reported a new norditerpenoid alkaloid acoseptine **60** from the roots of *A. septentrionale* Koelle (synonymous with *A. lycoctonum*).[50] Acoseptine **60** has a carbonyl at C-7 thus the carbon-carbon bond, which usually occurs at C-7 and C-17, changes to occur at C-8 and C-17. Norditerpenoid alkaloids of plants in *Aconitum* are generally the aconitine-type and non-arylated aconitine-type. However, some compounds from *A. monticola*[51] classified as the lycoctonine-type like swatinine **56** and acovulparine **57**.



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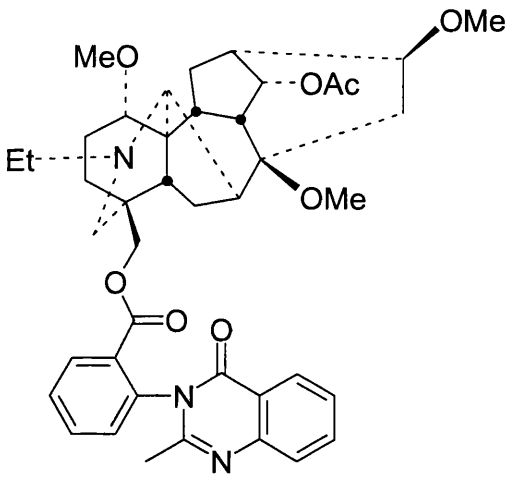

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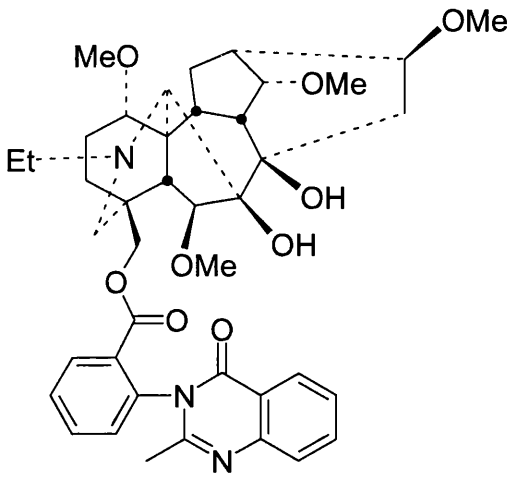
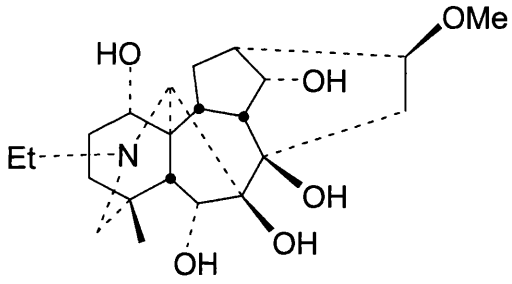


62

Shim and co-workers reported two new norditerpenoid alkaloids along with ten known compounds from the roots of *A. pseudo-laeve* var. *erectum*.[52] The two new alkaloids were assigned as 14-*O*-acetyl-8-*O*-methyl-18-*O*-2-(2-methyl-4-oxo-4*H*-quinazoline-3-yl)benzoyl-cammaconine **61** and 18-*O*-2-(2-methyl-4-oxo-4*H*-quinazoline-3-yl)benzoyllycoctonine **62**. The aryl group attached to C-18 of 18-*O*-2-(2-methyl-4-oxo-4*H*-quinazoline-3-yl)benzoyl lycoctonine **62** was unusual. Like swatinine **56** and acovulparine **57**, it is very unusual for *N*-deethylmethyllycaconitine **45** and 18-*O*-2-(2-methyl-4-oxo-4*H*-quinazoline-3-yl)benzoyllycoctonine **62** to be found in *Aconitum*.

1.3.3. Norditerpenoid alkaloids from *Consolida*

In the genus *Consolida*, Pelletier et al reported a new norditerpenoid alkaloid, consolarine **63** and three known compounds from the aerial parts of *C. armeniaca* (Stapf. ex Huth.) Schröd.[53] From the seeds of *C. ambigua*, Pelletier and Desai isolated a new norditerpenoid alkaloid conambine **64**.[54]



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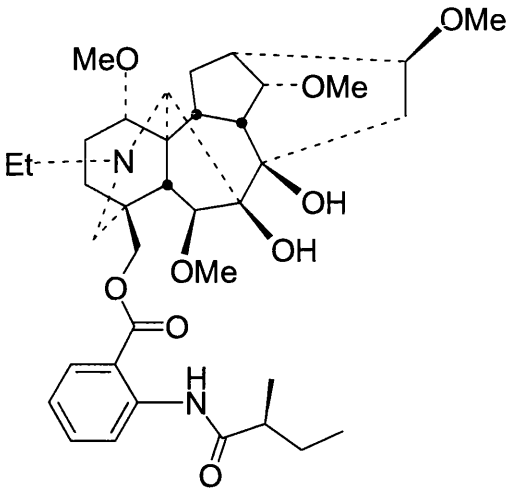

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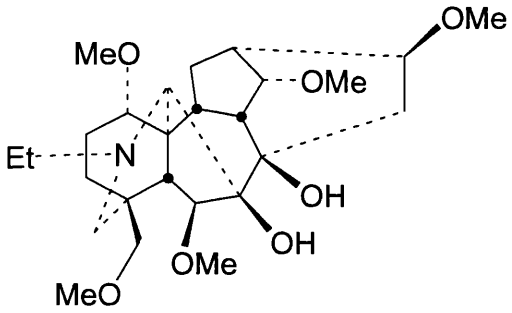

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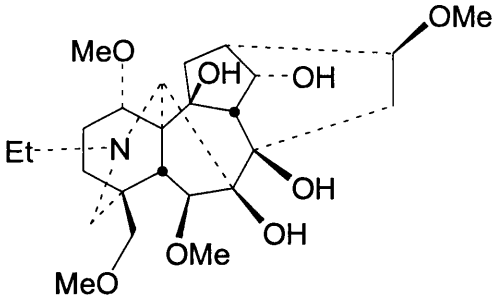
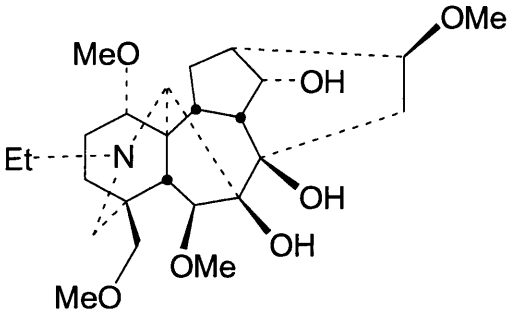


66



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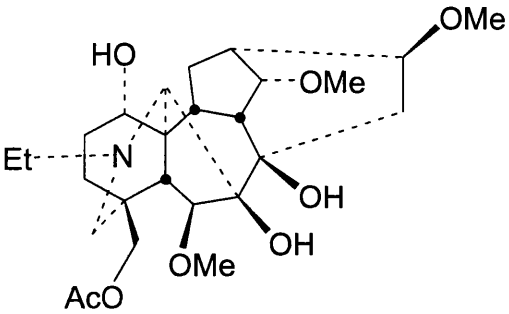
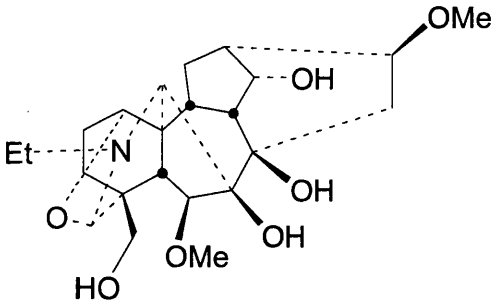


68

Mericli and co-workers reported three norditerpenoid alkaloids of the lycoctonine type, delphatine **65**, delcaroline **66** and browniine **67**, and three diterpenoid alkaloids from the aerial parts of *C. olopetala* (Boiss.) Hayek.[55] Gavín and co-workers reported seven new norditerpenoid alkaloids: 1-*O*-demethyltricornine **68**, the ether 1-*O*,19-didehydro-takaosamine **69**, 14-*O*-demethyldelboxine **70**, 8-*O*-methylconsolarine **71**, 14-*O*-deacetyl-pubescenine **72,** 14-*O*-benzoyltakaosamine **73** and 18-*O*-benzoyl-14-*O*-deacetyl-18-*O*-demethylpubescenine **74**, and 37 known lycoctonine-type norditerpenoid alkaloids isolated from the aerial parts of *C. orientalis* (Gay) Schrödinger subs. *orientalis*.[56]

1-*O*,19-Didehydrotakaosamine **69** presented the unusual structure forming an ether ring (oxygen atom bonded with C-1 and C-19, thus creating an additional substituted amino-acetal ring. 14-*O*-Demethyldelboxine **70** has an epoxide ring at C-3 and C-4 and lost C-18. Like lappaconitine **26**, 14-*O*-demethyldelboxine **70** might be considered as a bisnor-diterpenoid alkaloid. 14-*O*-Benzoyltakaosamine **73** and 18-*O*-benzoyl-14-*O*-deacetyl-18-*O*-demethylpubescenine **74** are acylated lycoctonine type though 14-*O*-benzoyl takaosamine **73** has benzoylation at C-14 more commonly found in aconitine type alkaloids. In the whole plants of the same species, Hohmann and co-workers reported a new norditerpenoid alkaloid, 14-deacetyl-18-demethylpubescenine **75**, and a new diterpenoid alkaloid.[57] 8-*O*-Methylconsolarine **71**, 14-*O*-deacetylpubescenine **72,** 18-*O*-benzoyl-14-*O*-deacetyl-18-*O*-demethylpubescenine **74**, and 14-deacetyl-18-demethylpubescenine **75** have different stereochemistry from other lycoctonine type alkaloids having C-6 substitution on the α-face.



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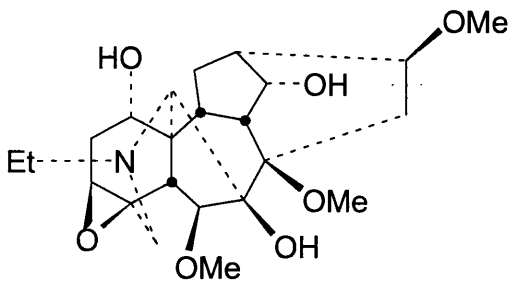

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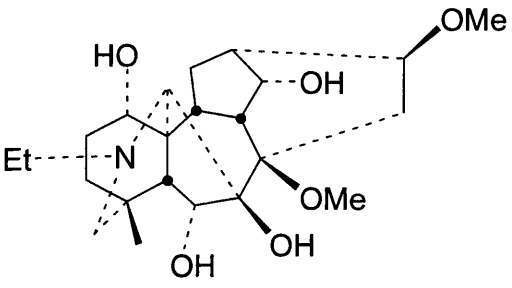

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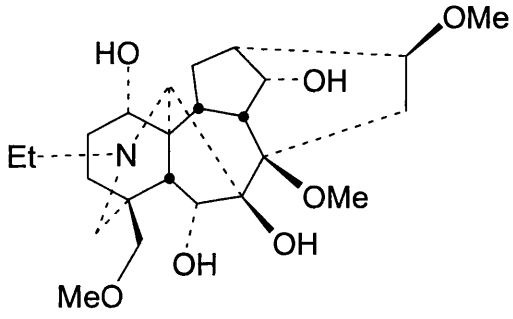

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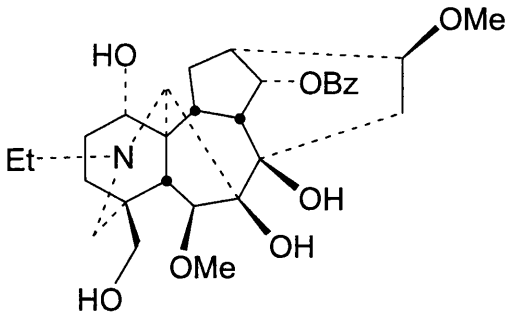

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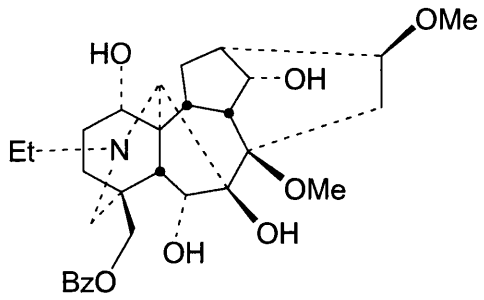

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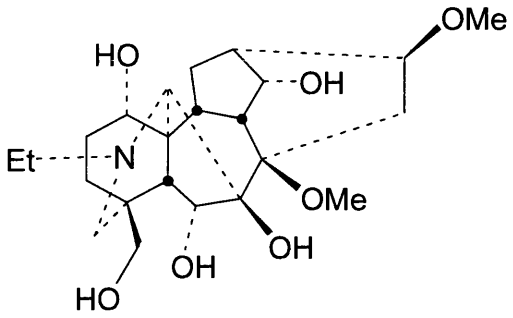
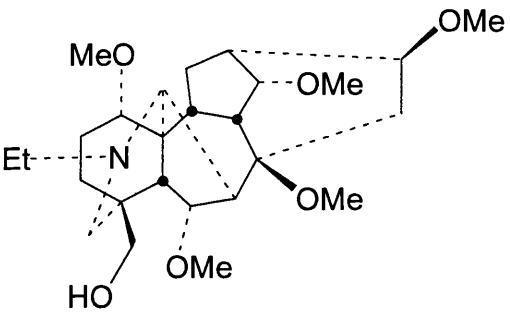


75

Ulubelen and co-workers reported two new aconitine-type alkaloids, hoheconsoline **76** and consolinine **77**, and one known lycoctonine-type alkaloid, lycoctonine, from the aerial parts of *C. hohenackeri* (Boiss.) Grossh. (synonymous with *Aconitella hohenackeri*, *Delphinium hohenackeri*) collected from eastern Turkey.[58] In general, alkaloids from *Consolida* are of the lycoctonine type, thus it was unusual to find hoheconsoline **76** and consolinine **77** in *Consolida*.



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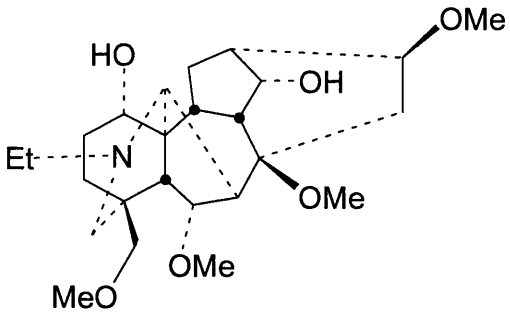
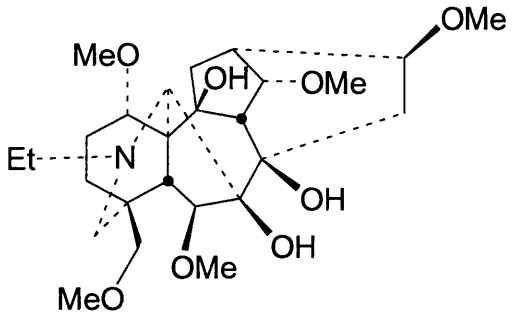


77

1.3.4. Norditerpenoid alkaloids from *Delphinium*

In the genus *Delphinium*, Bracher investigated norditerpenoid alkaloids from several *Delphinium* species (*D. dissectum* Huth, *D. excelsum* Reichenb, *D. grandiflorum* L. and *D. triste* Fisch) and isolated, eleven known and three new norditerpenoid alkaloids, 18-*O*-methyldelterine **78**, 10-hydroxynudicaulidine **79**, and 10-hydroxymethyllycaconitine **80**.[59]

Both *O*-methyldelterine **78** and 10-hydroxynudicaulidine **79** were isolated from the aerial parts of *D. excelsum* Reichenb and 10-hydroxymethyllycaconitine **80** from the aerial parts of *D. dissectum* Huth and *D. excelsum* Reichenb. Pelletier and co-workers reported known MLA 4 and deltaline **5** from the aerial parts of *D. cheilanthum* Fisch.[26] Furthermore, they isolated a new norditerpenoid alkaloid, delbruninol **81**, and six known compounds from the whole plants of *D. brunonianum* Royle colleted in the southwestern parts of China.[60]



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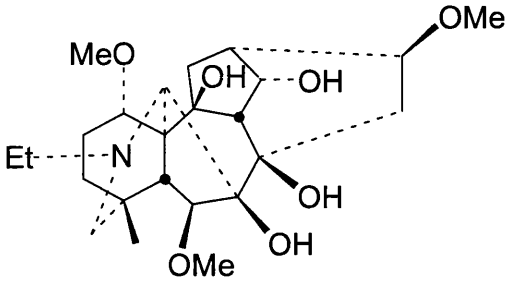

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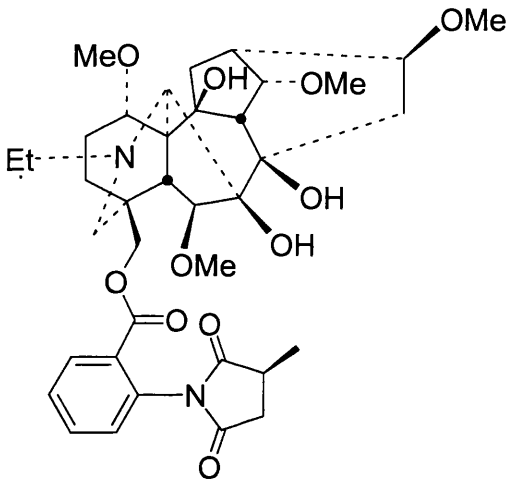

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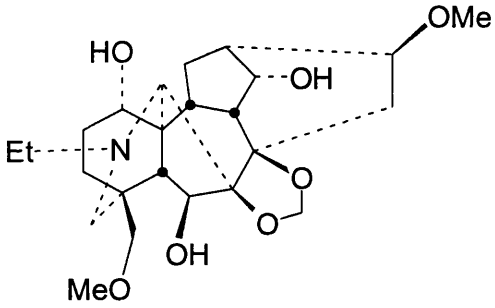

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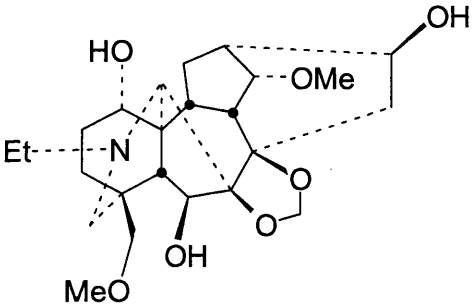

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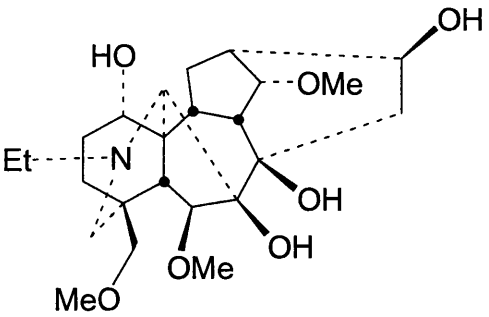
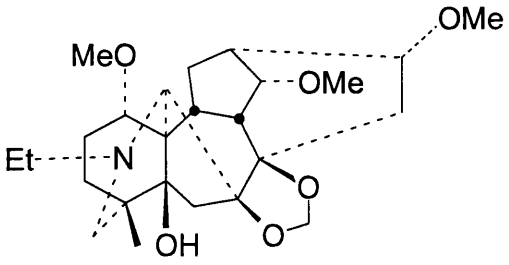


83

Salimov isolated a new norditerpenoid alkaloid named delcorinine **82** from the aerial parts of *D. corymbosum* collected during flowering in Kazaknstan.[61] Lin and co-workers reported a new norditerpenoid alkaloid, 16-demethyldelsoline **83**, and five known compounds from the aerial parts of *D. fangshanense* W.T. Wang collected in the northern part of China.[62]



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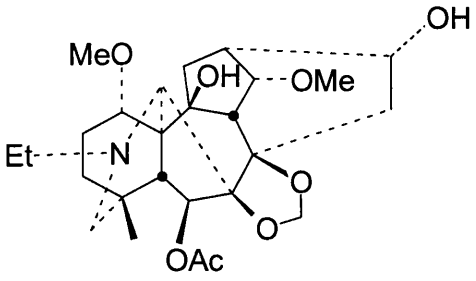

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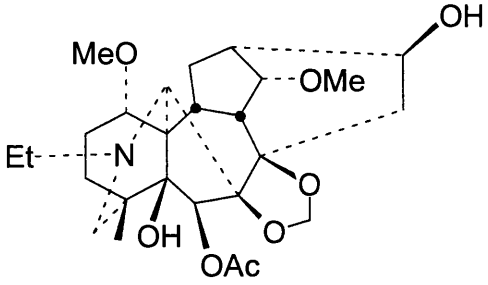

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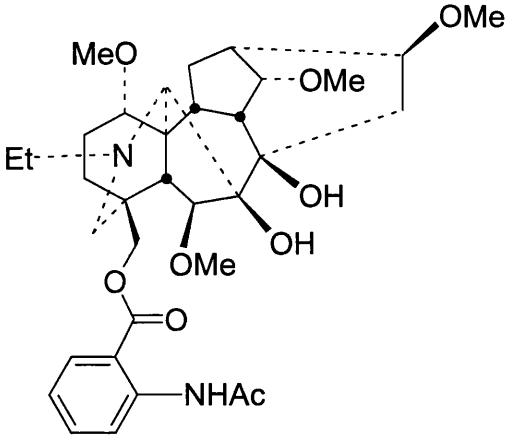

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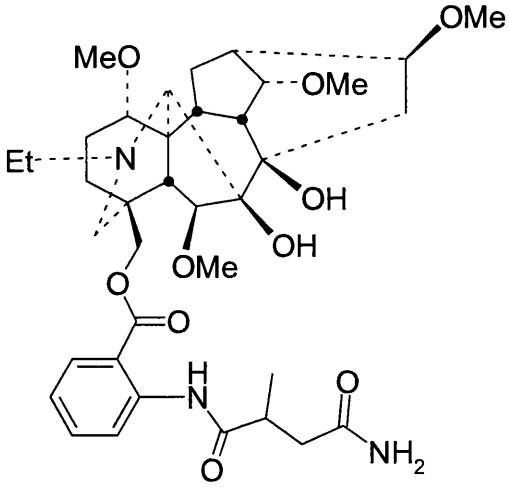

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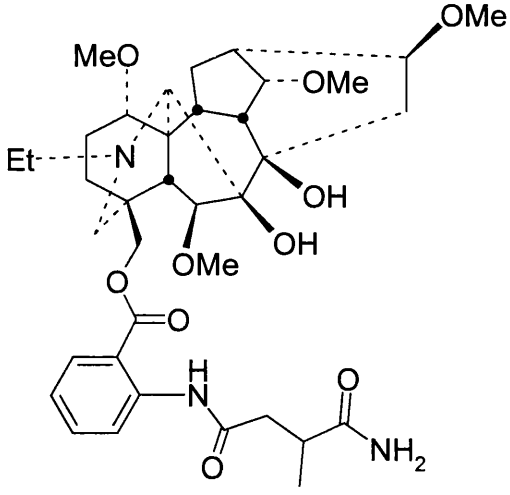

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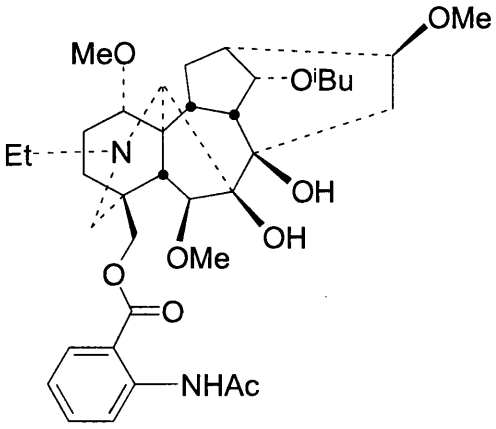
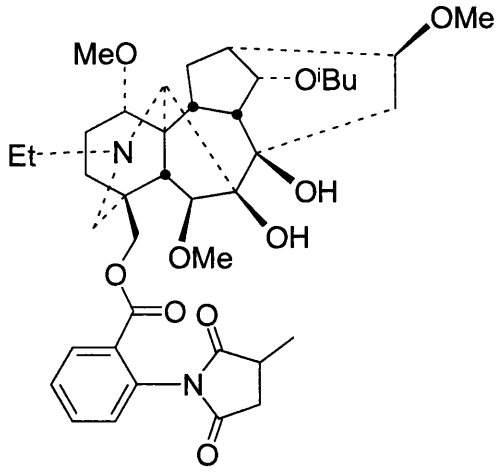
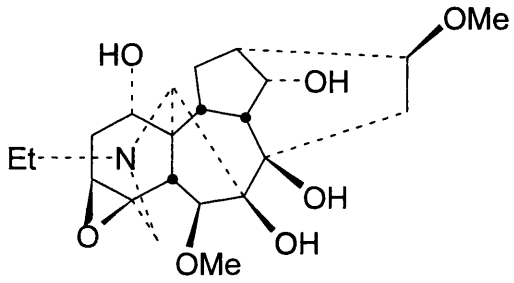


90



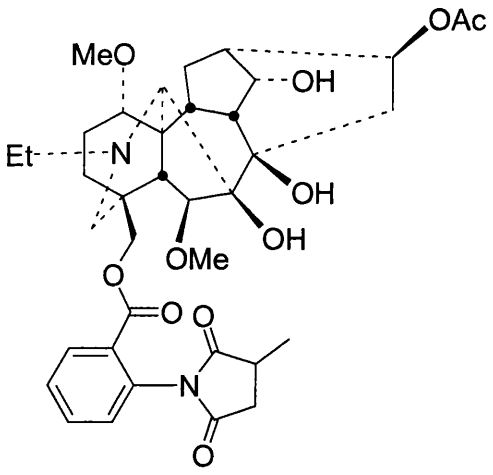
91

Shaheen and co-workers reported three new norditerpenoid alkaloids, nordhagenine A **84**, nordhagenine B **85** and nordhagenine C **86**, along with a known alkaloid from the aerial parts of *D. nordhagenii*.[63, 64] They suggested that nordhagenine A **84** and nordhagenine B **85** had substitution at C-16 on the α-face, different from other lycoctonine and aconitine type alkaloids. However, the published ORTEP diagrams of nordhagenine A **84** and nordhagenine B **85** showed they had C-16 substitution on the β-face. Mericli and co-workers reported six known lycoctonine type norditerpenoid alkaloids, gigactonine **27** and lycoctonine **6**, anthranoyllycoctonine (inuline) **7**, *N*-acetyldelectine **87**, delsemine A **88** and delsemine B **89** and a diterpenoid alkaloid from the aerial parts of *D. schmalhausenii*.[65] From the roots of *D. stapeliosum* Katz and Shrestha isolated three new norditerpenoid alkaloids, 14-deacetyl-14-isobutyrylnudicauline **90**, 14-deacetyl-14-isobutyrylajadine **91**, and 14-demethyltuguaconitine **92** and nine known alkaloids.[66]



92

Gardner and co-workers isolated three new arylated lycoctonine-type alkaloids, bearline **93**, 14-acetylbearline **94**, and 16-deacetylgeyerline **95**, from the aerial parts of *D. nuttallianum* Pritz.[67, 68] Yunusov reported that a new norditerpenoid alkaloid, 16-demethoxy-methyl-lycaconitine **96**, from the roots of *D. cuneatum*.[69]



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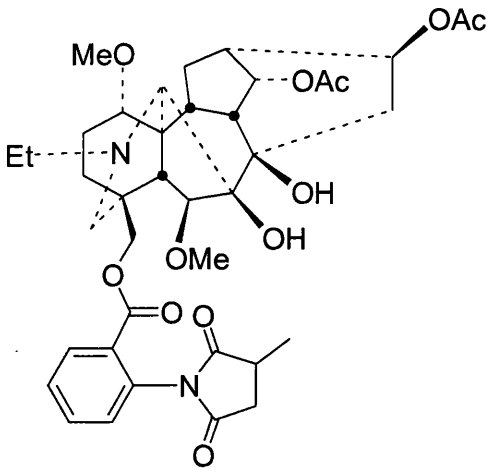

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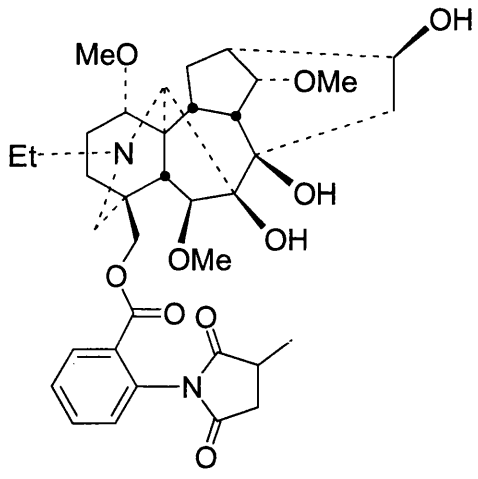

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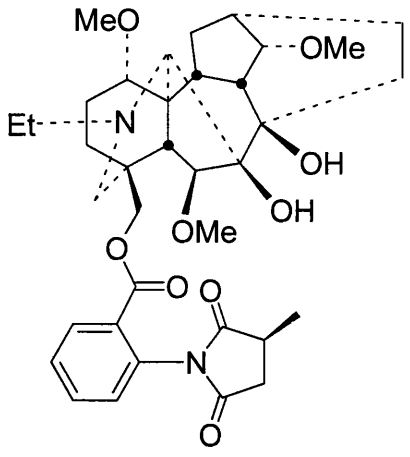

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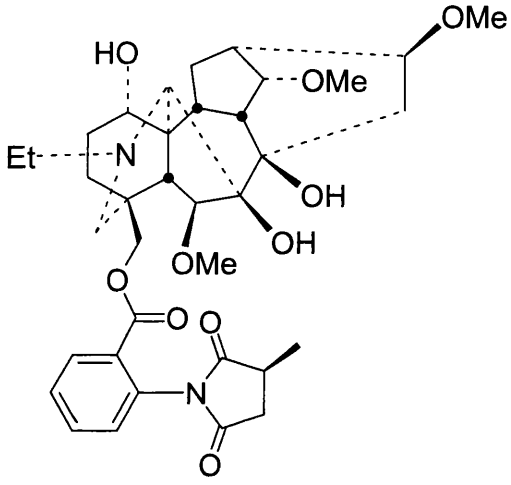

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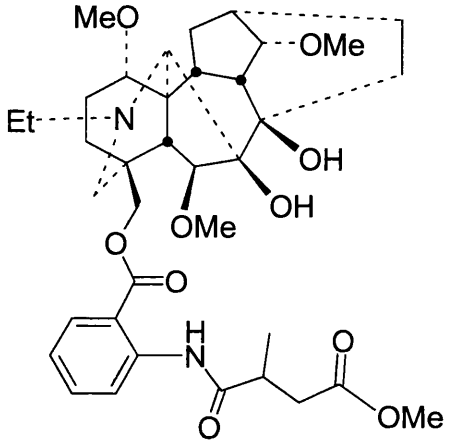

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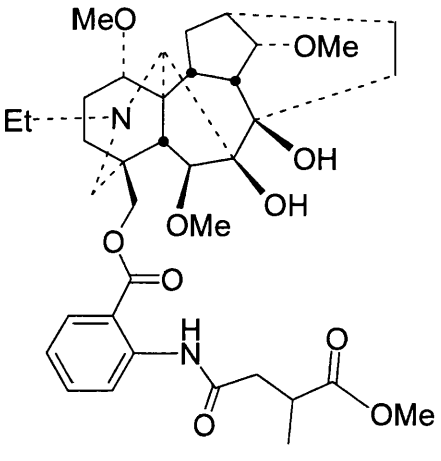

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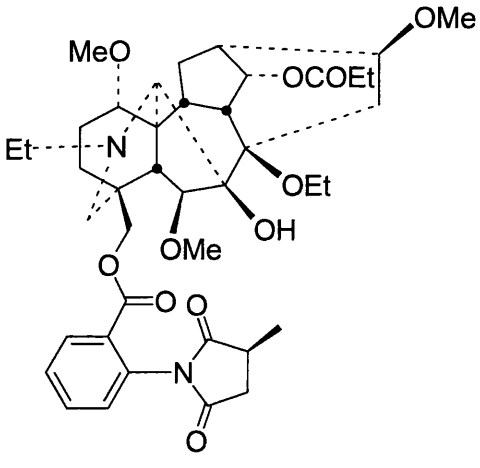
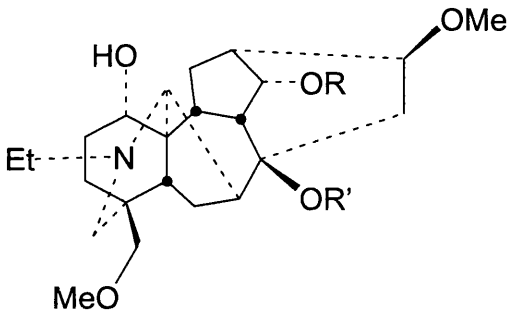


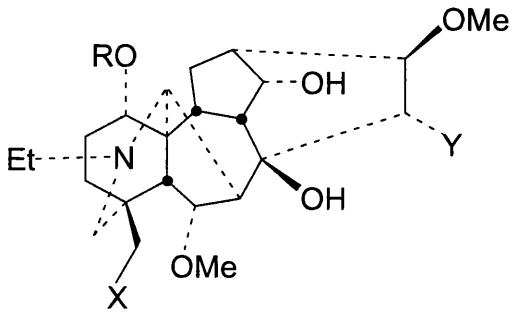
100

Gabbasov and co-workers reported a new norditerpenoid alkaloid, uraline **97**, from aerial parts of *D. uralense* Nevski.[70] From the same species, Khairitdinova and co-workers reported a regioisomeric mixture of two new norditerpenoid alkaloids 16-demethoxy-delavaine (A **98** and B **99**).[71] Furthermore, the same group also reported known alkaloids

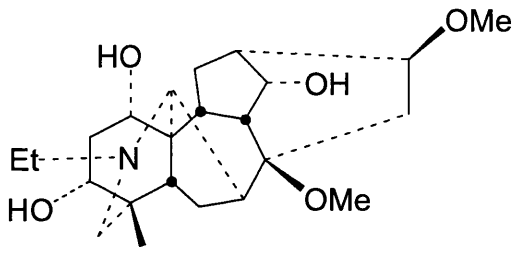
and the new norditerpenoid alkaloid alpinine **100** from the aerial part of *D. alpinum*.[72] Ulubelen and co-workers isolated a new norditerpenoid alkaloid, 8-acetylcondelphine **101**, and three known aconitine-type alkaloids, isotalatizidine **102**, condelphine **103**, and senbusine C **104** from the aerial parts of *D. pyrimadale* collected from the Swat district of Pakistan.[73] Furthermore, they reported a new norditerpenoid alkaloid, royleinine **105**, and three known compounds, isotalatizidine **102**, condelphine **103**, and senbusine C **104**, from *D. roylei* Munz.[74] They also reported two new norditerpenoid alkaloids, crispulidine **106** and delphicrispuline **107**, and six known compounds from the aerial parts of *D. crispulum* Rupr. (synonymous with *D.speciosum* Bieb var. *linearilobum* Trautv.).[75] These compounds are aconitine-type alkaloids and it is unusual for them to be found in *Delphinium*. With its C-18 skeleton, delphicrispuline **107** might be considered to be a bisnorditerpenoid alkaloid.



101 R = R' = Ac
102 R = R' =H
103 R = Ac, R' = H



104 R = H, X = OMe, Y = OH
105 R = Me, X = Y = H



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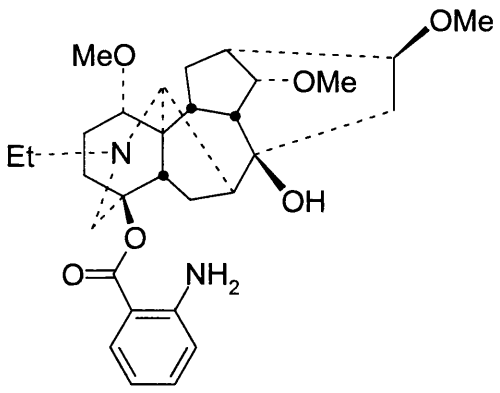


107

The structures of the new alkaloids detailed above were determined using spectroscopic techniques. However, many alkaloids were studied by X-ray analysis.[76-79] In summary, in the last seven years, more than eighty new norditerpenoid alkaloids with complex ring substitution patterns have been isolated. With newer separation techniques, natural products decorated with more sensitive functional groups are being purified and characterized, and this trend looks set to continue.

1.4. Biological activities and modes of action

1.4.1. *Aconitum*

Aconite is now found wild in a few parts of England, mainly in the western counties and also in South Wales, but can hardly be considered truly indigenous. It was very early introduced into England, being mentioned in all the English vocabularies of plants from the tenth century downwards, and in Early English medical recipes. Aconite is the trivial name for most of species in *Aconitum* and some species in other genera. It was derived from Greek meaning whetstone. Depending on the region, the Western aconite is the species generally cultivated in gardens, though nearly all the species are worth growing as ornamental garden flowers, the best perhaps being *A. napellus*, both white and blue, *A. paniculatum, A. japonicum and A. autumnale*. All grow well in shade and under trees. Japanese Aconite (syn. *Aconitum chinense*) is regularly imported in considerable quantities. It used formerly to be ascribed to *A. fischer* Reichb., but is now considered to be derived from *A. uncinatum*, var. *Faponicum* Regel. and possibly also from *A. volubile* Pallas. Nepal aconite consists of the root of *A. laciniatum* Staph. It is also called Bikh or Bish. Indian Aconite root was formerly attributed to *A. ferox* Wall., but now is considered to be derived from *A. chasmanthum* Staph. and *A. spicatum*. Russian aconite refers to *A. orientale*. However, winter aconite, *Aeranthis hyemalis*,which is poisonous in all parts of the plant, is not a true aconite, but in the genus *Eranthis*.[80]

The common Monkshood, *A. napellus*, was considered to be of therapeutic and toxicological importance. It has a short underground stem, from which dark-colored tapering roots descend. The crown or upper portion of the root gives rise to new plants. When touched to one's lip, the juice of the aconite root produces a feeling of numbness and tingling. The roots of *A. ferox* supply the Indian (Nepal) poison. It contains large quantities of the alkaloid pseudaconitine, which is a deadly poison. *A. palmatum* yields another of the poisons. The root of *A. luridum*, of the Himalaya, is said to be as virulent as that of *A. ferox* or *A. napellus*. Several species of *Aconitum* have been used as arrow poisons. The Minaro in Ladakh use *A. napellus* on their arrows to hunt ibex, while the Ainus in Japan used a species of *Aconitum* to hunt bear. The Chinese also used *Aconitum* poisons both for hunting and for warfare.[81]

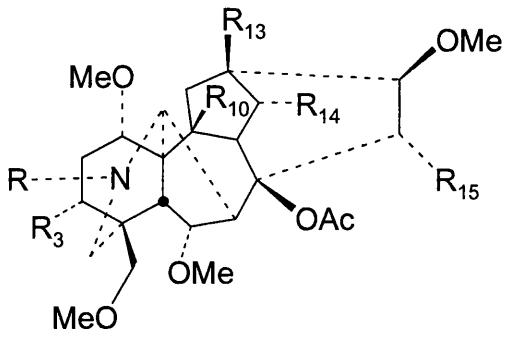
In folklore, aconite has even been ascribed with powers relating to werewolves and other lycanthropes, either to repel them, relating to the use of aconite in poisoning wolves and

other animals, or to induce their lycanthropic condition, as aconite was often an important ingredient in witches' ointments. Aconite is also said to make a person into a werewolf (or to kill them) if it is worn, smelled, or eaten. A Canadian film actor, Andre Noble, died of aconitine poisoning on July 30, 2004 after accidentally ingesting it. Aconite was reportedly found in toxicology samples from the former Pakistan cricket coach Bob Woolmer, but his death was later confirmed to be from natural causes.[82]

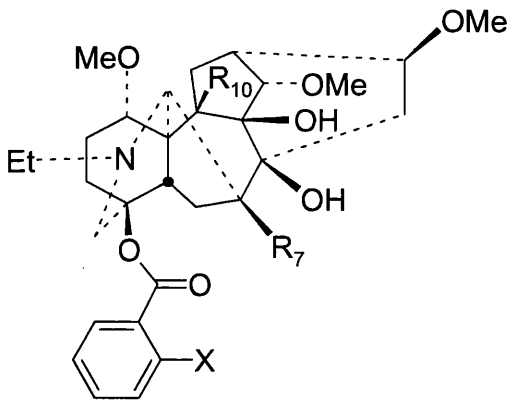
Aconite has long been used in the traditional medicine of India and China. In Ayurveda the herb is used to enhance penetration in small doses and to increase pitta. Pitta is one of the manifestations of elemental forces in the physical body, but not physical substances in themselves and pitta analogues to the fire element. However, more frequently the herb is detoxified according to the samskaras process and studies, cited in the detoxification section below show that it no longer possesses active toxicity. Aconite from the rhizome of *A. heterophyllum* is one ingredient of Tribhuvankirti, an Ayurvedic preparation for treating a "cold in the head" and fever.[83] It is used in traditional Chinese medicine as a treatment for Yang deficiency, "coldness" general debilitation. The herb is considered hot and toxic, thus it is prepared in extremely small prepared doses. More frequently ginger processed aconite, of lower toxicity, "fu zi" is used. Aconite was mixed with *Patrinia* and *Coix*, in a famous treatment for appendicitis described in a formula from the Jingui Yaolue (ca. 220 A.D.). Aconite was also described in Greek and Roman medicine by Theophrastus, Dioscorides, and Pliny, who most likely prescribed the Alpine species *A. lycoctonum*. The herb was cultivated widely in Europe, probably reaching England before the tenth century, where it was farmed with some difficulty, but it came to be widely valued as an anodyne, diuretic, and diaphoretic.[80] In the nineteenth century much aconite was imported from China, Japan, Fiji, and Tonga, with a number of species used to manufacture alkaloids of varying potency but generally similar effect, most often used externally and rarely internally. Effects of different preparations were standardized by testing on guinea pigs.[84]

In Western medicine preparations of aconite were used until just after the middle of the 20th century, but it is no longer employed as it has been replaced by safer and more effective drugs and treatments. The root is alterative, anaesthetic, antiarthritic, antitussive, deobstruent, diaphoretic, diuretic, sedative, and stimulant. This is a very poisonous plant and should only be used with extreme caution and under the supervision of a qualified practitioner. The 1911 British Pharmaceutical Codex regarded the medical uses and toxicity of aconite root or leaves to be virtually identical to that of purified aconitine. Aconite first stimulates and later paralyses the nerves of pain, touch, and temperature if applied to the

skin or to a mucous membrane; the initial tingling therefore gives place to a long-continued anaesthetic action. Great caution was required, as abraded skin could absorb a dangerous dose of the drug, and merely tasting some of the concentrated preparations available could be fatal. The local anaesthesia of peripheral nerves can be attributed to at least eleven alkaloids: aconitine **12**, 3-acetylaconitine **108**, beiwutine **109**, bulleyaconitine **110**, nagarine **111**, penduline **112**, lappaconitine **26**, *N*-deacetylfinaconitine **113**, *N*-deacetyllappaconitine **114**, *N*-deacetylranaconitine **115**, and ranaconitine **116**, with varying potency and stability.[85]



	R	R_3	R_{10}	R_{13}	R_{14}	R_{15}
aconitine **12**	Et	OH	H	OH	OBz	OH
3-acetylaconitine **108**	Et	OAc	H	OH	OBz	OH
beiwutine **109**	Me	OH	OH	OH	OBz	OH
bulleyaconitine **110**	Et	H	H	OH	OAs	H
nagarine **111**	Et	OH	OH	OH	OBz	OH
penduline **112**	Et	H	H	H	OBz	OH



	R_7	R_{10}	X
lappaconitine **26**	H	H	NHAc
N-deacetylfinaconitine **113**	OH	OH	NH_2
N-deacetyllappaconitine **114**	H	H	NH_2
N-deacetylranaconitine **115**	OH	H	NH_2
ranaconitine **116**	OH	H	NHAc

External uses of aconite included treatment of ordinary facial or trigeminal neuralgia, rheumatism, and dental periostitis. Internal uses were also pursued, to slow the pulse, as a sedative in pericarditis and heart palpitations, and well diluted as a mild diaphoretic, or to reduce feverishness in treatment of colds, pneumonia, quinsy, laryngitis, croup, and asthma due to exposure. Taken internally, aconite acts very notably on the circulation, the respiration, and the nervous system. The pulse is slowed, the number of beats per minute being actually reduced, under considerable doses, to forty, or even thirty, per minute. The blood-pressure synchronously falls, and the heart is arrested in diastole. Immediately before arrest, the heart may beat much faster than normally, though with extreme irregularity, and in the lower animals the auricles may be observed occasionally to miss a beat, as in poisoning by veratrine and colchicum. The action of aconitine on the circulation is due to an initial stimulation of the cardio-inhibitory centre in the medulla oblongata (at the root of the vagus nerves), and later to a directly toxic influence on the nerve-ganglia and muscular fibres of the heart itself. The fall in blood-pressure is not due to any direct influence on the vessels. The respiration becomes slower owing to a paralytic action on the respiratory centre and, in warm-blooded animals, death is due to this action, the respiration being arrested before the action of the heart. Aconite further depresses the activity of all nerve-terminals, the sensory being affected before the motor. In small doses, it therefore tends to relieve pain, if this is present. The activity of the spinal cord is similarly depressed. The pupil is at first contracted, and afterwards dilated. The cerebrum is totally unaffected by aconite, consciousness and the intelligence remaining normal to the last. The antipyretic action which considerable doses of aconite display is not specific but is the result of its influence on the circulation and respiration and of its slight diaphoretic action.

The aconite is very toxic. The poisoning was general taken by an oral administration. It should, however, be noted that aconitine may be easily absorbed through the skin, and poisoning may occur through this route simply by picking the leaves without the use of gloves; the toxin in the sap is absorbed through the skin.[86]

Aconitine is a potent neurotoxin that locks tetrodotoxin sensitive sodium channels in an open configuration. Pretreatment with barakol, 10 mg/kg IV the compound is isolated from the leaves of *Cassia siamea* Lam, reduces the incidence of aconitine-induced ventricular fibrillation and ventricular tachycardia, as well as mortality. Tetrodotoxin 5 μg/kg IV also had the same effect.[87] The protective effects of barakol are probably due to the prevention of intracellular sodium ion accumulation.

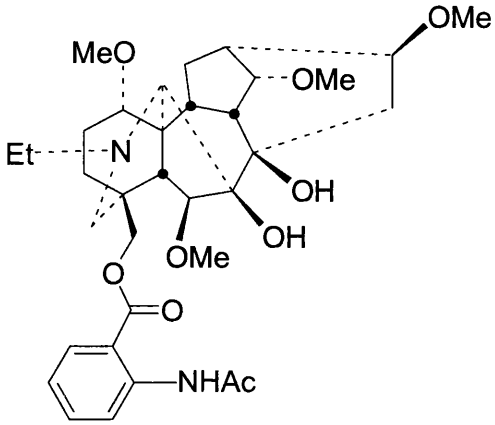
The physiological antidotes are atropine and digitalin or strophanthin, which should be injected subcutaneously in maximal doses. Alcohol, strychnine, and warmth must also be employed. There are methods of processing aconite to reduce toxicity in both Chinese medicine and ayurveda. In Chinese medicine, the traditional pao zhi or preparation of aconite is to steam with ginger in a fairly elaborate procedure. Due to the variable levels of toxicity in any given sample of the dried herb, there are still issues with using it. Most, but not all, cases of aconite toxicity in Taiwan were due to the consumption of unprocessed aconite.[88]

According to an article by the Indian scientists Thatte and co-workers, crude aconite is an extremely lethal substance.[83] However, the science of Ayurveda looks upon aconite as a therapeutic entity. Crude aconite is always processed i.e. it undergoes "samskaras" before being utilized in the Ayurvedic formulations. This study was undertaken in mice, to ascertain whether 'processed' aconite is less toxic as compared to the crude or unprocessed one. It was seen that crude aconite was significantly toxic to mice (100% mortality at a dose of 2.6 mg/mouse) whereas the fully processed aconite was non-toxic (no mortality at a dose even 8 times as high as that of crude aconite). Furthermore, all the steps in the processing were essential for complete detoxification.

1.4.2. *Delphinium*

Many species are cultivated as garden plants, with numerous cultivars having been selected for their denser, more prominent flowers. All parts of the plant are very poisonous, causing vomiting when eaten, and death in larger amounts. In small amounts, extracts of the plant have been used in herbal medicine. Gerard reported that drinking the seed of larkspur was thought to help against the stings of scorpions, and that other poisonous animals could not move when covered by the herb, but does not believe it himself.[89] Pliny the Elder and Grieve showed that the seeds can be used against parasites, especially lice and their nits in the hair. A tincture is used against asthma and dropsy. The juice of the flowers, mixed with alum, gives a blue ink. The plant was associated with Saint Odile and in popular medicine used against eye-diseases. It was one of the herbs used on the feast of St. John and as such warded against lightning. In Transylvania, it was used to keep witches from the stables, probably because of its blue color. *Delphinium* extracts have been used as medicines for many centuries.[90] These extracts have been used in different cultures for a wide variety of ailments, such as: sedatives, emetics, anthelmintics, analgesic balms and muscle relaxants and also in treating jaundice, dropsy, postencephalic parkinsonism, spinal arachnoditis and

disorders of the spleen. It is believed that the compounds present in these herbal remedies are major factor to cure these effects and so these traditional medicines may have implications for the design of modern-day drugs, in particular, with respect to the treatment of stroke, epilepsy and other forms of neurodegeneration. MLA **4** occurs in many *Delphinium* species, but despite its names, has so far not been isolated from any species of *Aconitum*. MLA shows much more action on the skeleton neuromuscular system than on the cardiovascular system. Kuzovkov and Bocharnikova noted similar properties associated with the alkaloids: elatine **3**, delsemines A and B (**88**, **89**), and ajacine **117** (also known as *N*-acetylanthranoyl lycoctonine or *N*-acetylinuline).[90]



117

These properties are the curare-like properties which inherent in esters of lycoctonine or amino alcohols like it, esterified with *N*-acylated anthranilic acid. Dozortseva reported the pharmacology of MLA, but studied as its hydroiodide salt, mellictine, and found that IV administration of the drug to mice resulted in temporary general depression and disturbed motor coordination.[90] No abnormalities were observed in cardiac rhythm. It was also found to be active after stomach-tube and rectal administration. Delsemine is produced by the action of ammonia on MLA. As such it is usually an artifact of the isolation procedure, but there is some evidence that delsemine may occur naturally.[91] Gubanow reported that delsemine is a neuromuscular blocker and a hypotensive agent and it has been used clinically in the Soviet Union as a substitute for tubocurarine in surgery.[92] Lycaconitine has been found in both *Aconitum* and *Delphinium* species.[93] It was found to have little effect on the heart rate of anesthetized rabbits in doses up to 5 mg/kg IV.[94] Dozortseva reported that the pharmacology of elatine seems to differ little from that of MLA, except that MLA exhibits slightly higher potency.[95] Elatine has been used in the Soviet Union for the treatment of neurological disorder.[96] In comparison with tubocurarine, elatine proved to be seven to eight times less toxic, but it claimed to have a five-to-six times wider therapeutic

spectrum.[97] Jacyno noted that inuline had some potency as a neuromuscular blocking reagent.[98] Lycoctonine caused a sudden fall in blood pressure when give IV to cats in doses of 2-5 mg/kg.[90] Jacyno reported that lycoctonine had no effect on the electrically induced contractions of the isolated guinea pig ileum at concentrations up to 10^{-5} M.[98] Parenteral administration of delsoline to mice caused weakness in the muscles of the extremities, clonic convulsion, and respiratory depression, with death resulting from asphyxiation.[90]

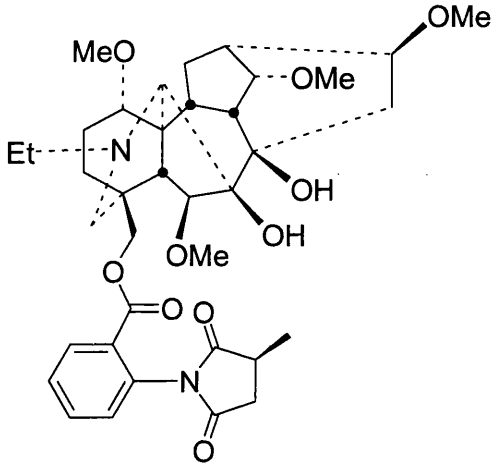
Many studies of *Delphinium* toxicity relate to "larkspurs" growing on North American ranges. Larkspur is the common and trivial name of some species *Delphinium* and *Consolida*. A small group of distinct wild *Delphinium* species namely *D. glaucum, D. barbeyi* and *D. occidentale* that occur on North America mountains and rangelands are referred to locally as tall larkspur. Larkspurs, especially tall larkspur, are a significant cause of cattle poisoning on rangelands in the western United States. Larkspurs are more common in high-elevation areas and contain numerous diterpenoid alkaloids. Intoxication results from neuromuscular paralysis, as nicotinic acetylcholine receptors in the muscle and brain are blocked. Clinical signs include laboured breathing, rapid and irregular heartbeat, muscular weakness, and collapse. Toxic alkaloid concentrations generally decline in tall larkspurs with maturation, but alkaloid concentration varies over years and from plant to plant, and is of little use for predicting consumption by cattle. Knowledge of toxic alkaloid concentration is valuable for management purposes when cattle begin to eat larkspur. Cattle generally begin consuming tall larkspur after flowering racemes are elongated, and consumption increases as larkspur matures. Weather is also a major factor in cattle consumption, as cattle tend to eat more larkspurs during or just after summer storms. Management options that may be useful for livestock producers include conditioning cattle to avoid larkspur (food aversion learning), grazing tall larkspur ranges before flowering (early grazing) and after seed shatter (late grazing), grazing sheep before cattle, herbicidal control of larkspur plants, and drug therapy for intoxicated animals. Some potentially fruitful research avenues include examining alkaloid chemistry in low and plains larkspurs, developing immunologic methods for analyzing larkspur alkaloids, developing drug therapy, and devising grazing regimes specifically for low and plains larkspur.

Tall larkspur poisoning of cattle is a serious problem on western US rangelands.[99] Single oral doses of tall larkspur ranging from 1.5 to 3 g/kg body weight were administered to steers. These doses caused clinical signs of muscular tremors and collapse. Physostigmine was administered iv, ip or sc at 0.04 to 0.08 mg/kg body weight when animals were sternally or laterally recumbent. Physostigmine given iv rapidly reversed the larkspur toxicity. Serial

injections of physostigmine were generally necessary to reverse acute toxicity. Administration of physostigmine to grazing animals poisoned on larkspur was also effective. Physostigmine can be effective treatment for intoxicated cattle consuming tall larkspurs.

1.4.3. Modes of action

Even though the aconitine-type and lycoctonine-type alkaloids possess the same skeleton, they differ in several functional groups, stereochemistry and significantly in modes of action. Aconitine **12** (aconitine-type alkaloid) and MLA **4** (lycoctonine-type alkaloid) are well known compounds for having high toxicity.



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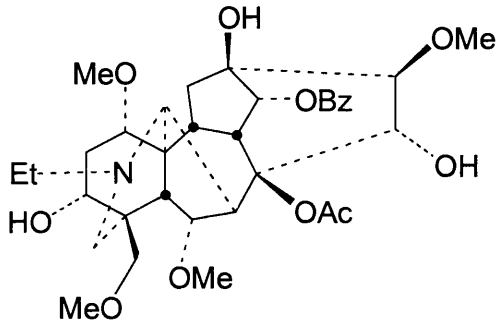


12

MLA **4** is a competitive nicotinic antagonist with approximately 100-, 1,000-, and 10,000-fold higher affinity for α7 nAChR, compared with α3β2, α4β2, and muscle nAChR respectively.[100-102] MLA is a competitive postsynaptic inhibitor of the neurotransmitter acetylcholine, acting at nicotinic receptor sites in mammals. It also acts in the same way at cholinergic ganglia in sympathetic and parasympathetic nerves. The pharmacophore associated with disturbance of cholinergic neurotransmission is the lycoctonine skeleton itself. The pattern of oxygenation and the electronic nature of oxygen-bearing functionalities appear to determine the physiological manifestations of this disturbance. Jacyno concluded that the ester group in MLA provides secondary binding sites or aids in orientation and suggested that the key acetylcholine receptor recognition sites are the nitrogen and the C-8 oxygen atoms.[98] However, Blagbrough and co-workers demonstrated that hydrogen atom donation from C7 and/or C8 hydroxyl functional groups, in forming a putative hydrogen bond, is not an absolute requirement for the binding of MLA to α7 nAChR. so suggested that the ester carbonyl oxygen in MLA could donate a lone pair of

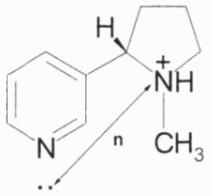
electrons to form a hydrogen bond at the ligand binding site and, finally, indicated the importance of the methylsuccinimido for nicotinic potency of MLA.[103]

Aconitine **12** shows neurotoxicity principally as a consequence of its abilities to interact with excitable membranes to hold open voltage-gated sodium channels following an action potential, so that prolonged depolarisation results. To consider structure activity relationships, the key structural features of the aconitine-like norditerpenoid alkaloids in receptor-binding properties possibly do not include the nitrogen atom. It is the relative positioning of the 16β-methoxyl, 15α-hydroxyl, 8β-acetoxy oxygen (or 14α-benzoyloxy oxygen) and possibly the methyl of the acetyl group that enhances aconitine with the ability to interact with the receptors.[90] The aconitine-like alkaloids are esters and the removal of the ester functions results in a significant drop in their toxicity and complete loss of neurotoxicity of the parent alkaloids. Furthermore, Blagbrough and co-workers showed that the addition of a 2-(methylsuccinimido)benzoyl sidechain to *O*-demethylated aconitine abolished Na^+ channel activation and conferred nanomolar affinity for brain binding sites, presenting a potent nicotinic ligand. This is important for the design of nicotinic subtype-selective drugs.[104]

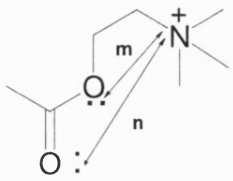
1.4.4. Nicotinic pharmacophore

Acetylcholine acts at two structurally and physiologically different receptor sites in the mammalian nervous system. These receptors were defined by the ligands that act selectively at each acetylcholine receptor (AChR) subtype and are the nicotinic and muscarinic acetylcholine receptors, nAChR and mAChR respectively.

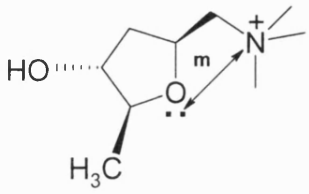
By conformational analysis, Beers and Reich demonstrated (Figure 1.4) that the distance (4.4 Å) from the positively charged nitrogen atom to the lone-pair electron on the oxygen atom in the ring of muscarine is the same as the distance from the positive charged nitrogen atom to the lone-pair electron on the ester oxygen atom in the ester acetylcholine.[105] Furthermore, the distance (5.9 Å) from the protonated nitrogen atom to the (orthogonal) lone-pair electrons on the nitrogen atom in the pyridine ring of nicotine is the same as the distance from the positively charged nitrogen atom and the lone-pair electrons on the oxygen atom of the carbonyl group of acetylcholine.



S-L-(-)-Nicotine



Acetylcholine



cis-L-(+)-Muscarine

Figure 1.4 Beers-Reich pharmacophore model. n is the distance from the positive charge of a nitrogen atom (e.g. in pyrrolidine) to a lone-pair of electrons of any heteroatom forming a hydrogen bond for recognition at nAChR, and m is the distance from the same positively charged *N*-atom to a lone-pair of electrons of any heteroatom forming a hydrogen bond for recognition at mAChR.

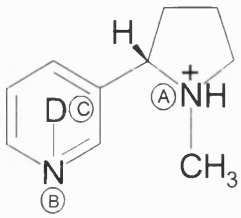
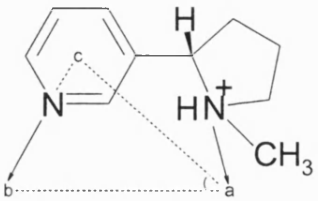


Figure 1.5 Sheridan nicotinic pharmacophore model

By an extension of conformational distance geometry techniques, Sheridan and co-workers showed (Figure 1.5) that the nicotinic pharmacophore is possibly the position of the cationic centre (A), an electronegative atom (B) and an atom (C) forming a dipole with B.[105] These atoms form an triangle with sides 4.8 Å (A-B), 4.0 Å (A-C), and 1.2 Å (B-C). For nicotine, D was a dummy atom along bond angle bisector and 1.2 Å from the pyridine nitrogen atom.



a-b = 7.3 -8.0 Å

a-c = 6.5 – 7.4 Å

Δbac = 30.4 – 35.8°

Figure 1.6 Novo Nordisk nicotinic pharmacophore model

Over recent years, no agreement has been reached as to the *N-N* (or *N-O*) distance for viable nicotinic ligands. Values ranging from 4.5 Å to more than 6 Å having been proposed, and

an analysis of this type, in isolation from other pharmacophore parameters, have to be treated with caution. Pettersson and co-workers have provided one of the most recent contributions to this area.[106] Their model also links *N-N* distances to activity, however, this is not regarded as either an essential or a unique pharmacophore component. Rather the Novo Nordisk nicotinic pharmacophore maps key interatomic distances and angles as shown in Figure 1.6. This vector-based model was developed and refined using a range of agonists with quite different molecular structures and binding activities. The Novo Nordisk model defines three site points: **a** relates to the cationic (usually protonated) nitrogen center, **b** correlates to the heteroatom (N or O) that is a hydrogen bond acceptor, and point **c** is the centre of a heteroatom ring or C=O. Sites **a** and **b** are located at 2.9 Å from the corresponding atoms and in the direction of the lone pair of electrons associated with each atom. The angle (Δbac) between the interatomic distance vectors **a-b** and **a-c,** and the distance **ab** and **ac** are the key parameters and idealised values are shown in Figure 1.6. This model indicates that it is not *N-N* (or *N-O*) distance that is a key determinant for nicotinic activity, rather it is the vectors to points **a** and **b** that are important. In this way, biologically active ligand classes with "short" and "long" *N-N* or *N-O* distances can be accommodated by this model. It can be seen from this example of nicotine that, for activity at the nicotinic receptor, the tertiary nitrogen atom must be protonated. The pK_a values of nicotine are 7.84 (*N*-methylpyrrolidine ring) and 3.04 (pyridine ring),[107] thus it is 73-92% protonated at physiological pH (7.4-6.8).[108] Similarly, the pK_a of the nitrogen atom of the alkaloids considered in these studies is relevant to their biological activity at nAChR.

1.5. Aims

A current and comprehensive review of norditerpenoid alkaloids from *Aconitum, Consolida,* and *Delphinium* with brief aspects of taxonomy, biological activities, and modes of action focus the introduction to this thesis. The aims of the research are: isolation and purification of norditerpenoid alkaloids from Delphinium cv Pacific Giant and *Aconitum lycoctonum* seeds, followed by characterisation chromatographically, spectroscopically, and by X-ray analysis where possible. Semi-syntheses of norditerpenoid alkaloids including: lycoctonine by basic hydrolysis of MLA, inuline by acidic hydrolysis of MLA and by esterification of lycoctonine, and elatine by methylenedioxy acetal formation of MLA are undertaken. Finally, a key physico-chemical parameter, pK_a, is measured for *N*-ethylpiperidine and MLA by a novel NMR approach as a proof of the applicability of such a spectroscopic technique.

2. Experimental

2.1. General techniques and instrumentation, chemicals, reagents, and solvents

Chemicals, reagents, and solvents were purchased from Acros, Fisher, and Sigma-Aldrich, all in GPR grade, unless otherwise stated. They were purified or dried as required according to the procedures in Perrin, Perrin and Amarego,[109] and Fieser and Fieser.[110] Dimethyl sulfoxide was stored over activated 4Å molecular sieves. Water refers generally to distilled water or to Milli-Q water for HPLC. Concentrated acids and bases are diluted in aqueous solutions unless otherwise stated and pH adjustments were monitored with pH paper.

Pacific Giant Delphinium seeds were obtained as two generous gifts (several kg) from Blackmore & Langdon, Pensford, UK. *Aconitum lycoctonum* was grown in Bath, UK, and harvested in the seeding stage in September 2005 and November 2006 respectively. The seeds of the plant material were air-dried at room temperature. The seeds of Delphinium cv Pacific Giant and *Aconitum lycoctonum* were ground using a grinder (Atomix, MSE).

Typical extraction or reaction work-ups ended with the organic solution being dried over anhydrous magnesium sulfate, filtered, and then evaporated to dryness under reduced pressure. Solvents were evaporated with a rotary evaporator (Büchi rotavapor) using a vacuum pump (Vacuubrand PC 2001 vario) with a pressure controller (Vacuubrand CVC 2^{II}) and a variable temperature water bath (Büchi waterbath).

Flash column chromatography was generally performed according to the procedure reported by Still and co-workers.[111] Column chromatography was routinely performed on silica gel 60 (purchased from Merck, 0.040-0.063 mm, 230-400 mesh ASTM, pH 6.5-7.5 for a 10% suspension) or on activated standard grade, neutral, acidic and basic (Brockmann I)alumina gels (purchased from Sigma-Aldrich, 58 Å, ~150 mesh) or by vaccum liquid chromatography (VLC). Solvent ratios are v/v unless otherwise stated.

Analytical TLC was performed on the commercial aluminium-backed plates pre-coated with silica GF_{254} 60 in 0.25 mm thickness and commercial aluminium-backed plates pre-coated with aluminium oxide GF_{254} 60 neutral purchased from Merck. TLC conditions were generally according to the procedures in Stahl.[112] TLC was used to determine a suitable mobile phase for isolation of the alkaloids from the extracts of *Delphinium* seeds and *Aconitum* seeds. The mobile system cyclohexane:chloroform:diethylamine (5:4:1) was used

to monitor the different pH extracts of *Delphinium* seeds, the extracts of *A. lycoctonum* seeds, and fractions obtained by column chromatography. The TLC plate was observed under short wave (254 nm) UV light and Dragendorff's reagent was used as a spray to visualize the alkaloids. Dragendorff's reagent was prepared by dilution of the stock solution (10 mL) with an aqueous solution of (+)tartaric acid (20% w/v). The stock solution was prepared from (+)tartaric acid (25 g) and bismuth oxynitrate (2.125 g) in water (100 mL). The solution was shaken for 1 hour and then potassium iodide (20 g) in water (50 mL) was added. The solution was allowed to stand for 24 h and then decanted.[113]

The high performance liquid chromatography (HPLC) instrument consisted of a solvent delivery system equipped with a Consta Metric 4100 constant volume pump, and monitored by a Jasco UV-1575 variable wavelength ultraviolet detector. HPLC detection was typically by UV absorbance at 275 nm, the λ_{max} of the anthranilate chromophore. Chromatogram traces were recorded on a Goerz Metramatt SE 120 recorder. All columns used were commercially pre-packed reversed phase HPLC columns, purchased from Phenomenex Inc.:
Analytical Column: Phenomenex Luna C18 5μ 150×4.60 mm;
Semi-preparative Column: Phenomenex Luna C18 10μ 250×10.00mm.

All the HPLC experiments were performed using isocratic elution. HPLC grade methanol was purchased from Fisher and filtered through a Whatman Nylon membrane prior to use. The acidic solution (0.1% formic acid) was filtered through Milli-Q Plus PF. Methanol was mixed with the 0.1% formic acid in the ratio methanol:0.1% formic acid (35:65). The aqueous mobile system was shaken vigorously and then degassed on a Decon ultrasonicater for 30 minutes before use. Samples were dissolved in pure organic solvent and filtered though a 17 mm diameter SYR Filter Nylon 0.45 μm before injection using a 20 μL loop for the analytical column and 100 μL loop for the semi-preparative column.

Ultraviolet (UV) spectra were measured in methanolic solutions using a Perkin-Elmer UV/VIS spectrophotometer Lambda EZ201. Infrared (IR) spectra were recorded from anhydrous KBr discs or NaCl plates using a Perkin-Elmer RX 1 FT-IR instrument.

Electron impact (EI) mass spectrometry (MS) was measured either on a VG AutoSpec instrument using EI ionization at 70eV, equipped with a Fisons autosampler in the Department of Chemistry, University of Bath, or on a Micromass Quattro II in the EPSRC National Mass Spectrometry Service Centre, University of Wales Swansea. Chemical

ionization (CI) MS was performed on a Micromass Quattro II in the EPSRC National Mass Spectrometry Service Centre, University of Wales Swansea using ammonia as the reagent gas. Electrospray ionization (ESI) MS was performed on a BRUKER DALTONICS micrOTOF in the Department of Pharmacy and Pharmacology, University of Bath.

NMR spectra were obtained on either a JEOL GX 270 (operating at 270 MHz for ^{1}H and 67.8 MHz for ^{13}C) or a Varian Mercury 400 MHz (operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer, in solutions in $CDCl_3$ (~0.5 mL) unless otherwise stated. All chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard at 0.0 ppm. COSY, DEPT, HMQC, HMBC spectra were all recorded using automated programmes. Coupling constants (*J*) are reported in Hertz (Hz, absolute values) and the multiplicities abbreviated as follows: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The abbreviation br (broad) is used to indicate significant broadening due to rapid exchange or unresolved fine coupling. The format used for reporting ^{1}H NMR spectra is: chemical shift (integration, multiplicity, coupling constant, and assignment).

Single crystals were analyzed at 150-152 K using graphite monochromated Mo(Kα) radiation and a Nonius Kappa CCD diffractometer by Dr. Mary Mahon in the Department of Chemistry, University of Bath. The structures were solved according to Sheldrick using SHELXS-97[114] and refined using SHELXL-97.[115]

2.2. Extraction of the seeds of Delphinium cv Pacific Giant

2.2.1. Extraction of the seeds of Delphinium cv Pacific Giant (1989 harvest)

Weighed ground seeds from 1989 (56 g) were defatted by Soxhlet extraction (5 cycles) with hexane (150 mL). The sample was further extracted with dichloromethane (5 cycles) followed by ethanol (5 cycles). The hexane extract was partitioned with 1M sulfuric acid (4 x 50 mL). The acidic layers were combined and then back partitioned with hexane (1 x 50 mL). The combined hexane layers were evaporated to give the hexane soluble fraction (not acidic soluble), a yellow oil (1.726 g). The pH of the acidic layer was adjusted to pH 7 with 2.5M sodium hydroxide and the solution was extracted with dichloromethane (4 x 100 mL). The combined organic extracts were washed with water (1 x 50 mL), dried ($MgSO_4$), and then evaporated to yield a pale yellow foam (24 mg). The dichloromethane extract was partitioned with 1M sulfuric acid (4 x 50 mL). The acidic layers were combined and then

back partitioned with dichloromethane (1 x 50 mL). The combined dichloromethane layers were evaporated to give the dichloromethane soluble fraction (not acidic soluble), a viscous brown oil (795 mg). The pH of the acidic layer was adjusted to pH 7 with 2.5M sodium hydroxide and the solution was extracted with dichloromethane (4 x 100 mL). The combined organic extracts were washed with water (1 x 50 mL), dried ($MgSO_4$), and then evaporated to yield a pale yellow foam (65 mg). The ethanol extract was evaporated to dryness under reduced pressure and the residue was extracted with 1M sulfuric acid (4 x 50 mL). The acidic layers were combined and then back partitioned with dichloromethane (1 x 50 mL). The residue after extraction was combined to the organic layer and then evaporated to give the ethanol soluble fraction (not acidic soluble), a dark brown viscous oil (261 mg). The pH of the acidic layer was adjusted to pH 7 with 2.5M sodium hydroxide and the solution was back extracted with dichloromethane (4 x 100 mL). The combined organic extracts were washed with water (1 x 50 mL), dried ($MgSO_4$), and then evaporated to yield a yellow foam (95 mg) (see Figure 2.1). The total of crude alkaloid materials extracted with three solvents was 153 mg (0.33% weight of seeds). TLC (cyclohexane:chloroform :diethylamine (5:4:1) detection by Dragendorff spray) showed no band of MLA ($R_f = 0.31$) (see section 2.3.1).

2.2.2. Extraction of the seeds of Delphinium cv Pacific Giant (1993 harvest)

Weighed ground seeds from 1993 (500 g) were defatted by Soxhlet extraction (5 cycles) with hexane (2 L). The sample was further extracted with dichloromethane (5 cycles) followed by ethanol (5 cycles). The hexane extract was concentrated to 300 mL and partitioned with 1M sulfuric acid (4 x 100 mL). The acidic layers were combined and then back partitioned with hexane (1 x 100 mL). The combined hexane layers were evaporated to give the hexane soluble fraction (not acidic soluble). The acidic layer was basified to pH 7 with 5M sodium hydroxide and the solution was extracted with dichloromethane (4 x 150 mL). The combined organic extracts were washed with water (1 x 100 mL), dried ($MgSO_4$), and then evaporated to yield a pale yellow foam (4.0 g). The dichloromethane extract was concentrated to 300 mL and partitioned with 1M sulfuric acid (4 x 100 mL). The acidic layers were combined and then back partitioned with dichloromethane (1 x 100 mL). The combined dichloromethane layers were evaporated to give the dichloromethane soluble fraction (not acidic soluble). The acidic layer was basified to pH 7 (measured with pH paper) with 5M sodium hydroxide and the solution was extracted with dichloromethane (4 x 150 mL). The combined organic extracts were washed with water (1 x 100 mL), dried ($MgSO_4$), and then evaporated to yield a yellow foam (1.8 g). The ethanol extract was

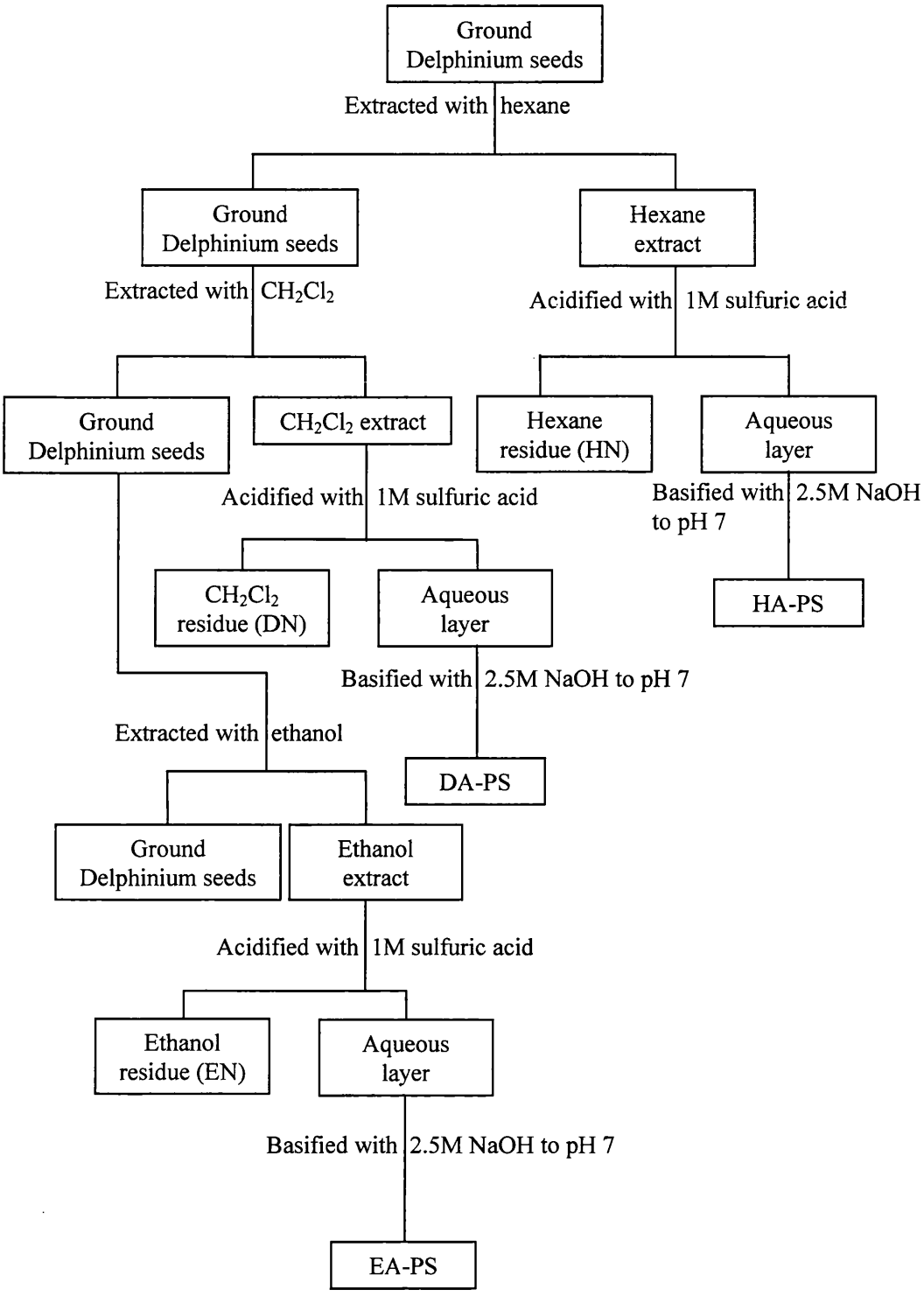


Figure 2.1 Extraction of the seeds of Delphinium cv Pacific Giant (1989 harvest)

evaporated to dryness under reduced pressure and the residue was extracted with 1M sulfuric acid (4 x 100 mL). The acidic layers were combined and then back partitioned with dichloromethane (1 x 100 mL). The residue after extraction was combined to the organic layer and then evaporated to give the ethanol soluble fraction (not acidic soluble). The acidic layer was basified to pH 7 with 5M sodium hydroxide and the solution was back extracted with dichloromethane (4 x 150 mL). The combined organic extracts were washed with water (1 x 100 mL), dried ($MgSO_4$), and then evaporated to yield a yellow foam (5.9 g). The total of crude alkaloid materials extracted with hexane, dichloromethane, and ethanol was 2.34 g (2.34% weight of seeds). TLC (cyclohexane:chloroform:diethylamine (5:4:1) detection by Dragendorff spray) showed eight bands for the crude hexane extract, eleven bands for the crude dichloromethane extract, and ten bands for the crude ethanol extract. Three crude extracts showed MLA ($R_f = 0.31$).

Weighed ground seeds from 1993 (500 g) were defatted by Soxhlet extraction (35 cycles) with hexane (2 L). The sample was further extracted with ethanol (30 cycles). The hexane extract was concentrated to 300 mL and partitioned with 1M sulfuric acid (4 x 100 mL). The acidic layers were combined and then back partitioned with hexane (1 x 100 mL). The combined hexane layers were evaporated to give the hexane soluble fraction (not acidic soluble), a yellow oil (117 g). The acidic layer was basified to pH 7 with 5M sodium hydroxide and the solution was extracted with dichloromethane (4 x 150 mL). The combined organic extracts were washed with water (1 x 100 mL), dried ($MgSO_4$), and then evaporated to yield a viscous yellow oil (13.7 g). The ethanol extract was evaporated to dryness under reduced pressure and the residue was extracted with 1M sulfuric acid (4 x 100 mL). The acidic layers were combined and then back partitioned with dichloromethane (1 x 100 mL). The residue after extraction was combined with the organic layer and then evaporated to give the ethanol soluble fraction (not acidic soluble), a viscous brown oil (6.9 g). The acidic layer was basified to pH 7 with 5M sodium hydroxide and the solution was back extracted with dichloromethane (4 x 150 mL). The combined organic extracts were washed with water (1 x 100 mL), dried ($MgSO_4$), and then evaporated to yield a yellow foam (6.8 g). The total of crude alkaloids extracted with hexane and ethanol was 20.5 g (4.1% weight of seeds). TLC (cyclohexane:chloroform:diethylamine (5:4:1) detection by Dragendorff spray) showed MLA ($R_f = 0.31$).

Weighed ground seeds from 1993 (20 g) were defatted by Soxhlet extraction (5 cycles) with hexane (150 mL). The sample was further extracted with chloroform (5 cycles). The hexane extract was partitioned with 0.5M sulfuric acid (4 x 50 mL). The acidic layers were

combined and then back partitioned with hexane (1 x 50 mL). The combined hexane layers were evaporated to give the hexane soluble fraction (not acidic soluble), a yellow oil (3.895 g). The acidic layer was basified to pH 7 with 5M sodium hydroxide and the solution was extracted with dichloromethane (4 x 100 mL). The combined organic extracts were washed with water (1 x 100 mL), dried ($MgSO_4$), and then evaporated to yield a pale yellow foam (31 mg). The chloroform extract was partitioned with 0.5M sulfuric acid (4 x 50 mL). The acidic layers were combined and then back partitioned with chloroform (1 x 50 mL). The combined chloroform layers were evaporated to give the chloroform soluble fraction (not acidic soluble), a viscous brown oil (921 mg). The acidic layer was basified to pH 7 with 5M sodium hydroxide and the solution was extracted with dichloromethane (4 x 100 mL). The combined organic extracts were washed with water (1 x 100 mL), dried ($MgSO_4$), and then evaporated to yield a yellow foam (52 mg). The total of crude alkaloids extracted with hexane and chloroform was 83 mg (0.43% weight of seeds). TLC (cyclohexane:chloroform: diethylamine (5:4:1) detection by Dragendorff spray) showed MLA ($R_f = 0.31$).

2.2.3. Extraction of the seeds of Delphinium cv Pacific Giant (1993 harvest) at different pH values

Weighed ground seeds from 1993 (500 g) were defatted by Soxhlet extraction (5 cycles) with hexane (2 L). The sample was further extracted with dichloromethane followed by ethanol (5 cycles). The hexane extract was concentrated to 300 mL and partitioned with 0.5M sulfuric acid (4 x 200 mL). The acidic layers were combined and then back partitioned with hexane. The combined hexane layers were evaporated to give the hexane soluble fraction (not acidic soluble). The pH of the acidic layer was adjusted to pH 4.6 with solid sodium bicarbonate, to pH 7.1 with solid sodium carbonate, and to pH 10.0 with 0.1M sodium hydroxide; and at each pH was back extracted with dichloromethane (5 x 100 mL). The pH of solution was measured by pH meter. At each pH, the combined organic extracts were washed with water (1 x 50 mL), dried ($MgSO_4$), and then evaporated. The dichloromethane extract was concentrated to 300 mL and partitioned with 0.5M sulfuric acid (4 x 200 mL). The acidic layers were combined and then back partitioned with dichloromethane. The combined dichloromethane layers were evaporated to give the dichloromethane soluble fraction (not acidic soluble). The pH of the acidic layer was adjusted to pH 4.6 with solid sodium bicarbonate, to pH 7.1 with solid sodium carbonate, and to pH 10.0 with 0.1M sodium hydroxide; and at each pH was back extracted with dichloromethane (5 x 100 mL). At each pH, the combined organic extracts were washed with water (1 x 50 mL), dried ($MgSO_4$), and then evaporated. The ethanol extract was

evaporated to dryness under reduced pressure and the residue was extracted with 0.5M sulfuric acid (4 x 200 mL). The acidic layers were combined and then back partitioned with dichloromethane. The residue after extraction was combined to the organic layer and then evaporated to give the ethanol soluble fraction (not acidic soluble). The pH of the acidic layer was adjusted to pH 4.6 with solid sodium bicarbonate, to pH 7.1 with solid sodium carbonate, and to pH 10.0 with 0.1M sodium hydroxide. At each pH, the solution was back extracted with dichloromethane (5 x 100 mL). At each pH, the combined organic extracts were washed with water (1 x 50 mL), dried ($MgSO_4$), and then evaporated (see Figure 2.2). The total of crude alkaloids extracted with hexane, dichloromethane, and ethanol and each at pH 4.6, 7.1, and 10.0 was 13.5 g (2.7% weight of seeds). TLC (cyclohexane:chloroform: diethylamine (5:4:1) detection by Dragendorff spray) showed that every fraction contained at least some MLA ($R_f = 0.31$) (see section 2.3.1).

2.3. Separation of crude extracts isolated from the seeds of Delphinium cv Pacific Giant

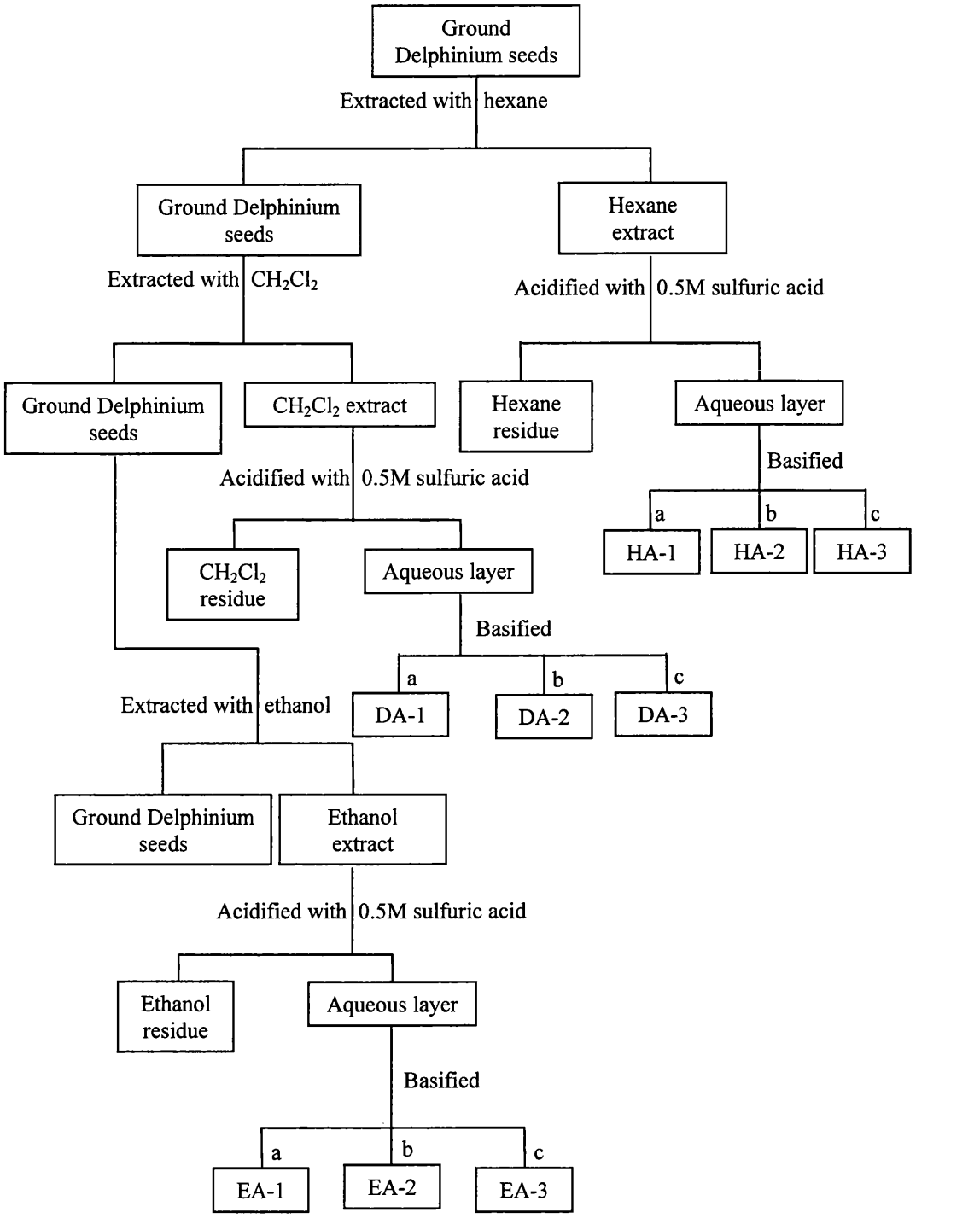
2.3.1. TLC of the extracts of Delphinium seeds

1989 harvest

Twelve samples were spotted on a silica TLC plate (20 cm x 20 cm), as follows:

1. Neutral substances extracted with hexane (HN)
2. Neutral substances extracted with dichloromethane (DN)
3. Alkaloids extracted with hexane (HA-MGR)
4. Alkaloids extracted with hexane (HA-PS)
5. Delpheline (authentic) (Delp)
6. Alkaloids extracted with dichloromethane (DA-MGR)
7. Alkaloids extracted with dichloromethane (DA-PS)
8. Methyllycaconitine (authentic) (MLA)
9. Alkaloids extracted with ethanol (EA-MGR)
10. Alkaloids extracted with ethanol (EA-PS)
11. Alkaloids extracted with 1M sulfuric acid (SAA)
12. Neutral substances extracted with ethanol (EN)

Four mobile phases were used to test for the most suitable system for flash column chromatography. The mobile phases were: DCM, DCM:methanol (90:10), DCM:methanol: conc. ammonia solution (90:10:0.5) and cyclohexane:chloroform:diethylamine (5:4:1). Each TLC tank of mobile phases contained two silica TLC plates. Four plates from each



a means basified with sodium bicarbonate and adjusted to pH 4.6 then extracted with CH_2Cl_2
b means basified with sodium carbonate and adjusted to pH 7.1 then extracted with CH_2Cl_2
c means basified with 0.1M sodium hydroxide and adjusted to pH 10.0 then extracted with CH_2Cl_2

Figure 2.2 Extraction of the seeds of Delphinium cv Pacific Giant (1993 harvest) at different pH

mobile phase were sprayed with 20% sulfuric acid and the other four plates of each mobile phase were sprayed with Dragendorff's reagent.

1993 harvest

Eleven samples were spotted on a silica TLC plate (20 cm x 20 cm), as follows:

1. Hexane soluble alkaloids extracted at pH 4.6 (HA-1)
2. Hexane soluble alkaloids extracted at pH 7.1 (HA-2)
3. Hexane soluble alkaloids extracted at pH 10.0 (HA-3)
4. Delpheline (authentic) (Delp)
5. Dichloromethane soluble alkaloids extracted at pH 4.6 (DA-1)
6. Dichloromethane soluble alkaloids extracted at pH 7.1 (DA-2)
7. Dichloromethane soluble alkaloids extracted at pH 10.0 (DA-3)
8. Methyllycaconitine (authentic) (MLA)
9. Ethanol soluble alkaloids extracted at pH 4.6 (EA-1)
10. Ethanol soluble alkaloids extracted at pH 7.1 (EA-2)
11. Ethanol soluble alkaloids extracted at pH 10.0 (EA-3)

The mobile system using to test for the flash column chromatography is cyclohexane: dichloromethane:diethylamine (5:4:1).

2.3.2. Vacuum Liquid Chromatography

The Vacuum Liquid Chromatography (VLC) apparatus used in these experiments was modified from that reported by Pelletier and co-workers[116] and consisted of a sintered-glass Buchner filter funnel over a B19 Quickfit joint and a specifically designed piece of glassware was connected to this joint. The glassware was composed of a 3-way tap, both arms were connected to B19 Quickfit joints each equipped with a side-arm for connection to a vacuum pump, where each side-arm was connected to a three-way stopcock used to control the vacuum provided by a vacuum pump. Round-bottomed flasks were used to collect the eluate from each arm of the apparatus in turn. A layer of TLC grade neutral alumina was packed on the frit and allowed to settle by gentle tapping under gravity. Then the vacuum was applied, and the adsorbent was compressed to a hard layer by suction. After the uniform and tight packing of the adsorbent, the vacuum was released. Hexane was poured quickly onto the surface of the adsorbent, and then the vacuum was reapplied. The column was then sucked to dryness, and the crude ethanol extract (1 g) from the seeds of *Delphinium* cv Pacific Giant dispersed on Celite was carefully introduced onto the surface

of the packing (without any vacuum). Enough solvent was used to cover completely the top surface of the adsorbent. Then vacuum was applied gently to draw the sample into the packing. The column was then developed under gentle vacuum with appropriate solvent mixtures in order of increasing polarity: hexane, diethyl ether in hexane (10-90% in 10% steps), diethyl ether, methanol in diethyl ether (2.5-15% in 2.5% steps and 15-40% in 5% steps), pulling the column dry between each fraction collected. The fractions were collected in round-bottomed flasks. After each fraction was collected, an appropriate solvent was added to the top of the column without vacuum until the surface was well covered. Then the vacuum was gently reapplied. Each fraction was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour.

2.3.3. Flash column chromatography

On flash silica gel

Crude ethanol extract (500 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on silica gel with mobile phases dichloromethane: methanol: conc. ammonia solution (97:3:0.25, 1 L) and dichloromethane:methanol: conc. ammonia solution (95:5:0.25, 1 L) in order of increasing polarity. Four fractions were obtained. TLC (cyclohexane:chloroform:diethylamine (5:4:1) detection by Dragendorff spray) of fraction 3 showed MLA ($R_f = 0.31$).

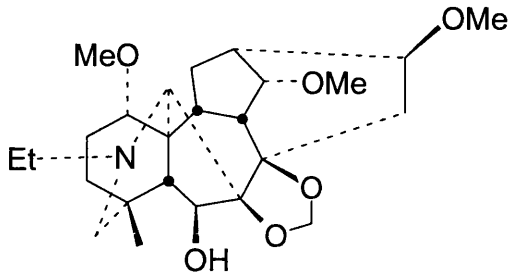
On neutral alumina

Crude ethanol extract (500 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on neutral alumina with mobile phase 2% methanol in dichloromethane (1 L). Three fractions were obtained. TLC (cyclohexane:chloroform: diethylamine (5:4:1) detection by Dragendorff spray) of fraction 2 showed MLA ($R_f = 0.31$).

On basic alumina

Crude ethanol extract (250 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on basic alumina with mobile phase 2% methanol in dichloromethane (500 mL). Four fractions were obtained. TLC (cyclohexane:chloroform: diethylamine (5:4:1) detection by Dragendorff spray) of fraction 2 showed MLA ($R_f = 0.31$). The elution was collected in test tubes, each contained approximately 10 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour.

2.3.4. Isolation of delpheline 1

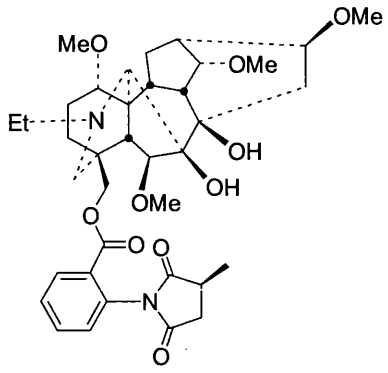


1

The crude hexane extracts at pH 7.1 and 10.0 were dissolved in dichloromethane and evaporated to dryness. The residue formed was dissolved in hot ethanol:hexane (1:1), and allowed to cool to room temperature over 16 h when crystals formed which were filtered and washed with cold ethanol:hexane (1:1). The crystals were identified as delpheline **1** by chromatography, NMR, HRMS, and X-ray diffraction of a single crystal.

TLC (cyclohexane:chloroform:diethylamine (5:4:1), detection by Dragendorff spray) showed $R_f = 0.45$. δ_H ($CDCl_3$) (400 MHz) 5.13 (1H, AB d, 1H of OCH_2O), 5.05 (1H, AB d, 1H of OCH_2O), 4.19 (1H, s, H-6), 3.71 (1H, m, H-14), 3.67 (1H, m, H-9), 3.43 [3H, s, C(14)OCH_3], 3.35 [3H, s, C(16)OCH_3], 3.34 (1H, br s, C(6)O*H*), 3.26 [3H, s, C(1)OCH_3], 3.25 (1H, m, H-16), 3.08 (1H, br s, H-17), 3.02 (1H, dd, J = 10 and 7, H-β-1), 2.77 (1H, dq, J = 7 and 12, 1H of NCH_2CH_3), 2.69 (2H, m, H-α-19 and 1H of NCH_2CH_3), 2.55 (1H, dd, J = 15 and 5, H-α-12), 2.49 (1H, dd, J = 15 and 9, H-α-15), 2.37 (1H, dd, J = 7 and 5, H-13), 2.24 (1H, d, J = 12, H-β-19), 2.21 (2H, m, H-α-2 and H-10), 2.07 (1H, m, H-β-2), 1.86 (2H, m, H-β-12 and H-β-15), 1.59 (1H, ddd, J = 13, 5, and 2, H-α-3), 1.26 (1H, m, H-β-3), 1.22 (1H, s, H-5), 1.06 (3H, t, J = 7, NCH_2CH_3) and 0.93 (3H, s, H_3-18); δ_C ($CDCl_3$) (100 MHz) 92.9 (OCH_2O), 92.4 (C-7), 84.1 (C-8), 83.0 (C-14), 8.27 (C-1), 81.8 (C-16), 79.2 (C-6), 63.6 (C-17), 57.8 [C(14)OCH_3], 57.6 (C-19), 56.6 (C-5), 56.3 [C(1)OCH_3], 55.6 [C(16)OCH_3], 50.7 (NCH_2CH_3), 50.5 (C-11), 47.7 (C-10), 40.4 (C-9), 37.8 (C-13), 36.9 (C-3), 33.9 (C-4), 33.4 (C-15), 28.2 (C-12), 26.7 (C-2), 25.4 (C-18), and 13.9 (NCH_2CH_3); $C_{25}H_{39}NO_6$ requires MW 449; HRMS: m/z of MH^+ $C_{25}H_{40}NO_6$ requires 450.2856, found 450.2843.

2.3.5. Isolation of methyllycaconitine 4



4

Crude dichloromethane extract at pH 4.6 (500 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on acidic alumina and mobile phases were: 40% diethyl ether in hexane, methanol in diethyl ether (2.5-10% in 2.5% steps then 30% and 40%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 15 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Six fractions were obtained and fraction 3 was identified as MLA **4** by chromatography, detailed NMR spectroscopy, and HRMS.

TLC (cyclohexane:chloroform:diethylamine (5:4:1), detection by Dragendorff spray) showed $R_f = 0.31$ (authentic MLA **4** $R_f = 0.31$). δ_H ($CDCl_3$) (400 MHz) 8.05 (1H, d, $J = 7$, H-6'), 7.70 (1H, t, $J = 7$, H-4'), 7.55 (1H, t, $J = 7$, H-5'), 7.30 (1H, d, $J = 7$, H-3'), 4.15 (2H, m, H-β-18 and H-α-18), 3.85 (1H, s, H-6), 3.60 (1H, dd, $J = 5.1$ and 5, H-14), 3.45 [3H, s, C(6)OCH_3], 3.42 [3H, s, C(14)OCH_3], 3.38 [3H, s, C(16)OCH_3], 3.28 [3H, s, C(1)OCH_3], 3.24 (1H, m, H-16), 3.10 (2H, m, H-9 and 1H of H-3"), 3.02 (1H, m, H-2"), 2.95 (3H, m, H-1, H-17, and 1 of NCH_2CH_3), 2.69 (2H, m, H-α-19 and 1H of NCH_2CH_3), 2.64 (1H, m, H-α-15), 2.56 (1H, m, 1H of H-3"), 2.50 (1H, m, H-α-12), 2.45 (1H, m, H-β-19), 2.35 (1H, m, H-13), 2.20-2.15 (2H, m, H-2), 1.98 (2H, m, H-10 and H-β-12), 1.78 (1H, m, H-α-3), 1.70 (2H, m, H-5 and H-β-15), 1.58 (1H, m, H-β-3), 1.47 (3H, m, H-5"), and 1.07 (3H, t, $J = 7$, NCH_2CH_3); δ_C($CDCl_3$) (100 MHz) 179.9 (C-1"), 175.8 (C-4"), 164.1 (C=O), 133.7 (C-4'), 133.1 (C-2'), 131.0 (C-6'), 130.1 (C-3'), 129.4 (C-5'), 126.9 (C-1'), 90.8 (C-6), 88.5 (C-7), 84.0 (C-1), 83.9 (C-14), 82.6 (C-16) 77.4 (C-8), 69.5 (C-18), 64.6 (C-17), 58.1 [C(6)OCH_3], 57.9 [C(14)OCH_3], 56.4 [C(16)OCH_3], 55.9 [C(1)OCH_3], 52.3 (C-19), 51.1 (NCH_2CH_3), 50.2 (C-5), 49.1 (C-11), 46.1 (C-10), 43.2 (C-9), 38.2 (C-13), 37.6 (C-4), 37.0 (C-3"), 35.2 (C-2"), 33.6 (C-15), 32.1 (C-3), 28.7 (C-12), 26.2 (C-2), 16.4 (C-5"), 14.1 (NCH_2CH_3); $C_{37}H_{50}N_2O_{10}$ requires MW 682; HRMS: m/z of MH^+ $C_{37}H_{51}N_2O_{10}$ requires 683.3544, found 683.3528.

2.3.6. Optimisation of MLA isolation

On acidic alumina

Repeating the above purification. Crude dichloromethane extract at pH 4.6 (500 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on acidic alumina and mobile phases were: diethyl ether, methanol in diethyl ether (2.5-10% in 2.5% steps then 30% and 40%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 15 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Seven fractions were obtained and fraction 5 was identified as MLA, but with some contamination.

On neutral alumina

Crude dichloromethane extract at pH 4.6 (500 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on neutral alumina and mobile phases were: 40% diethyl ether in hexane, diethyl ether, methanol in diethyl ether (2.5%, 5%, 10%, 30%, 40%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 15 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Seven fractions were obtained and fraction 3 was identified as MLA.

On basic alumina

Crude dichloromethane extract at pH 4.6 (1 g) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on basic alumina and mobile phases were: methanol in diethyl ether (2%, 5%, 10%, 30%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 10 mL for the middle fractions and 30 mL eluate for the early and late fractions. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Four fractions were obtained and fraction 2 showed MLA, but with some contamination.

On acidic alumina

Crude dichloromethane extract at pH 7.1 (500 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on acidic alumina and mobile phases were: methanol in diethyl ether (2%, 5%, 7%, 10%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 45 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined

according to the determination of their TLC behaviour. Six fractions were obtained and fraction 2 was identified as MLA, but with some contamination.

On basic alumina

Crude dichloromethane extract at pH 10.0 (180 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on basic alumina and mobile phases were: methanol in dichloromethane (1%, 2%, 5%, 10%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 10 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Eight fractions were obtained and fraction 6 was identified as MLA, but with some contamination.

Crude ethanol extract at pH 4.6 (1 g) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on basic alumina and mobile phases were: methanol in diethyl ether (2%, 5%, 10%, 30%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 10 mL eluate for the mobile phases 5% and 10% methanol in diethyl ether and 30 mL eluate for the mobile phases 2% and 30% methanol in diethyl ether, and methanol. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Six fractions were obtained and fractions 4 and 5 were identified as MLA, but with some contamination. Fractions 4 and 5 were combined and repurified by flash column chromatography on basic alumina and mobile phases were: methanol in diethyl ether (5%, 7.5%, 9%, 10%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 10 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Five fractions were obtained and fraction 2 was identified as MLA.

On neutral alumina

Crude ethanol extract at pH 4.6 (500 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on neutral alumina and mobile phases were: methanol in dichloromethane (0.5%, 1%, 2%, 5%, 10%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 10 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Four fractions were obtained and fraction 2 was identified as MLA.

Repeating the above purification. Crude ethanol extract at pH 4.6 (500 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on neutral alumina and mobile phases were: methanol in dichloromethane (1%, 2%, 5%, 10%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 10 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour.

On acidic alumina

Crude ethanol extract at pH 4.6 (500 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on acidic alumina and mobile phases were: methanol in diethyl ether (2%, 5%, 7%, 10%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 45 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Seven fractions were obtained and fraction 4 was identified as MLA, but with some contamination.

On basic alumina

Crude hexane extract at pH 4.6 (500 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on basic alumina and mobile phases were: methanol in diethyl ether (2.5%, 5%, 10%, 30%, 40%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 40 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. This process yielded eleven fractions.

Crude ethanol extract (1.5 g) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on basic alumina and mobile phases were: methanol in diethyl ether (2%, 4%, 5%, 7.5%, 10%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 45 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Six fractions were obtained and fraction 2 was identified as MLA.

On neutral alumina

Crude ethanol extract (500 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on neutral alumina and mobile phases were: dichloromethane, 2-propanol in dichloromethane (1%, 2%, 5%), in order of increasing

polarity. The elution was collected in test tubes, each contained approximately 45 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Three fractions were obtained and fraction 2 (from 5% 2-propanol in dichloromethane) was identified as MLA, but with some contamination which was further purified *vide infra* to yield delavaines A **119** and B **120**.

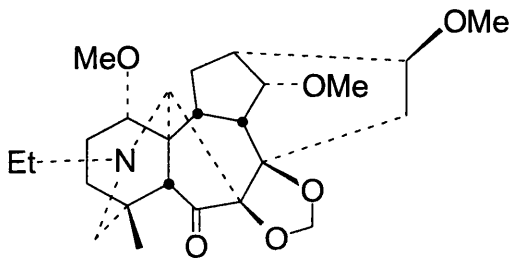
Repeating the above purification, but using methanol in diethyl ether not 2-propanol in dichloromethane. Crude ethanol extract (2 g) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on neutral alumina and mobile phases were: methanol in diethyl ether, (2.5%, 5%, 6%, 7.5%, 15%, 30%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 45 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Nine fractions were obtained and fraction 5 was identified as MLA with trace contamination.

On acidic alumina

Crude ethanol extract (300 mg) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on acidic alumina and mobile phases were: methanol in diethyl ether (2%, 5%, 7%, 10%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 45 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Six fractions were obtained and fraction 3 was identified as MLA with some contamination.

Of the methods tested, adsorption chromatography on neutral alumina eluted with a step gradient of methanol in diethyl ether gave the greatest yield of pure MLA free from contaminating alkaloids.

2.3.7. Isolation of pacinine 118



118

Crude ethanol extract (1 g) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on acidic alumina and mobile phases were: methanol in diethyl ether (2%, 5%, 7%, 10%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 45 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Twelve fractions were obtained, fraction 2 (from 2% methanol in diethyl ether) was identified as pacinine **118** and fraction 8 (from 5% methanol in diethyl ether) was identified as MLA **4**.

TLC (cyclohexane:chloroform:diethylamine (5:4:1), detection by Dragendorff spray) of fraction 2 showed one spot ($R_f = 0.80$), but no spot under UV light. δ_H ($CDCl_3$) (400 MHz) 5.51 (1H, AB d, 1H of OCH_2O), 5.07 (1H, AB d, 1H of OCH_2O), 3.62 (1H, m, H-14), 3.46 (1H, m, H-9), 3.38 [3H, s, C(14)OCH_3], 3.34 [3H, s, C(16)OCH_3], 3.30 [3H, s, C(6)OCH_3], 3.23 (1H, m, H-16), 3.11 (1H, br s, H-17), 3.03 (1H, dd, J = 10 and 7, H-β-1), 2.77 (1H, dq, J = 7 and 12, 1H of NCH_2CH_3), 2.69 (2H, m, H-α-19 and 1H of NCH_2CH_3), 2.55 (1H, dd, J = 15 and 5, H-α-12), 2.51 (1H, dd, J = 15 and 9, H-α-15), 2.37 (1H, dd, J = 7 and 5, H-13), 2.24 (1H, d, J = 12, H-β-19), 2.21 (2H, m, H-α-2 and H-10), 2.07 (1H, m, H-β-2), 1.86 (2H, m, H-β-12 and H-β-15), 1.59 (1H, ddd, J = 13, 5, and 2, H-α-3), 1.26 (1H, m, H-β-3), 1.24 (1H, s, H-5), 1.05 (3H, t, J = 7, NCH_2CH_3) and 0.91 (3H, s, H_3-18); $C_{25}H_{37}NO_6$ requires MW 447, HRMS: m/z of MH^+ $C_{25}H_{38}NO_6$ requires 448.2699, found 448.2687.

2.3.8. Attempted purifications of other alkaloids

Crude ethanol extract (1 g) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on silica gel and mobile phases were: methanol in diethyl ether (2.5%, 5%, 7.5%, 10%, 30%), and methanol in order of increasing polarity, each mobile phase was basified with conc. ammonia solution (1% v/v). The elution was collected in test tubes, each contained approximately 45 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Three fractions were obtained with relatively unsuccessful separation.

Crude ethanol extract (1.1 g) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on TLC grade alumina and mobile phases were: diethyl ether, methanol in diethyl ether (2%, 4%, 5%, 7.5%, 10%, 40%), and methanol in order of increasing polarity, each mobile phase was basified with conc. ammonia solution (1% v/v). The elution was collected in test tubes, each contained approximately 45 mL eluate. The

eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Eight fractions were obtained and fraction 6 was identified as MLA, but with trace contamination.

Crude dichloromethane extract (1.8 g) from the seeds of Delphinium cv Pacific Giant was purified by flash column chromatography on neutral alumina and mobile phases were: methanol in diethyl ether (2.5%, 5%, 7.5%, 15%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 45 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Six fractions were obtained and fraction 3 was identified as MLA, but with some contamination. Repeating this purification in order to obtain another sample of MLA for semi-synthetic studies. Crude dichloromethane extract (1 g) from the seeds of Delphinium cv Pacific Giant, purified by flash column chromatography on neutral alumina, gave eight fractions and fraction 5 was identified as MLA with trace contamination, sufficiently pure for hydrolysis.

After MLA was separated from the crude extracts, the fluorescent fractions which eluted before MLA from the crude dichloromethane extract were combined and then purified by flash column chromatography on neutral alumina and mobile phases were: cyclohexane, cyclohexane: dichloromethane (50:50), dichloromethane, and 10% methanol in dichloromethane in order of increasing polarity. The elution was collected in test tubes, each contained approximately 10 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Six fractions were obtained.

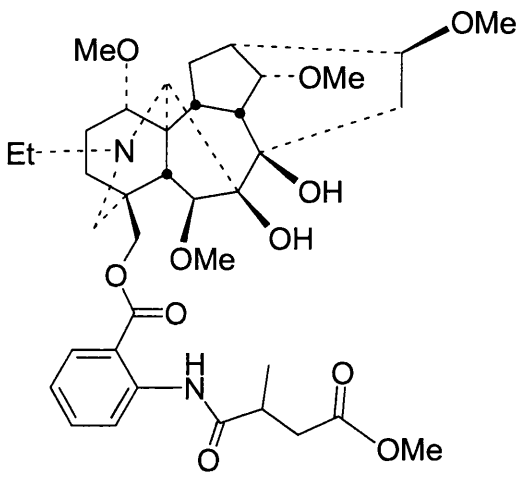
The fractions before MLA from the crude dichloromethane extract were combined and then purified by flash column chromatography on neutral alumina and mobile phases were: diethyl ether, methanol in diethyl ether (1%, 2%, 3%, 4%, 6%, 10%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 45 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Ten fractions were obtained.

The fractions after MLA from the crude dichloromethane extract were combined and then purified by flash column chromatography on neutral alumina and mobile phases were: methanol in diethyl ether (3%, 5%, 6%, 8%, 10%, 20%, 40%), and methanol in order of

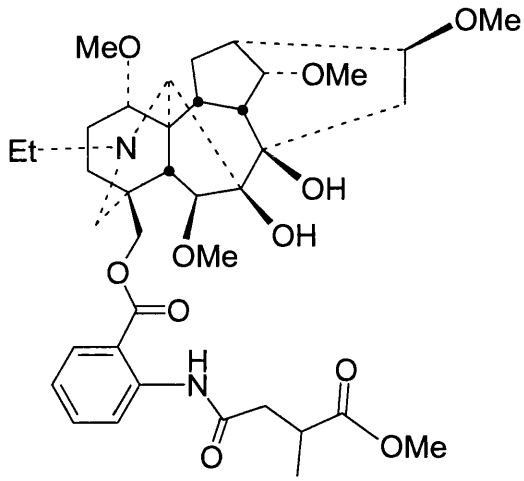
increasing polarity. The elution was collected in test tubes, each contained approximately 45 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Fourteen fractions were obtained.

The fractions before MLA from the crude ethanol extract were combined and then purified by flash column chromatography on neutral alumina and mobile phases were: methanol in diethyl ether (1%, 2%, 3%, 4%, 5%, 7%, 10%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 45 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Eleven fractions were obtained.

2.3.9. Isolation of delavaines A 119 and B 120



119

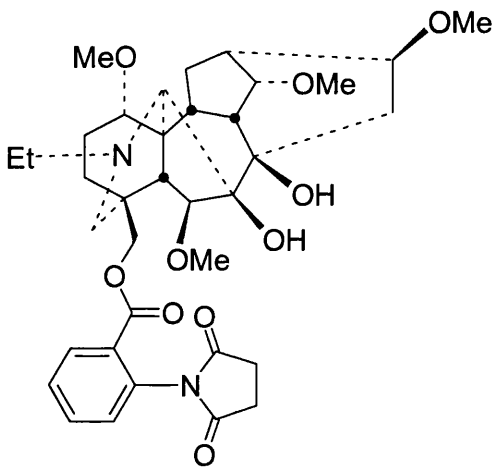


120

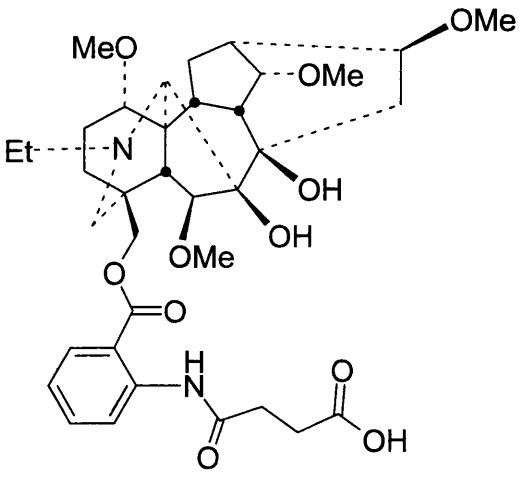
The fractions after MLA from the crude ethanol extract *vide supra* were combined and then purified further by flash column chromatography on neutral alumina and mobile phases were: methanol in diethyl ether (3%, 5%, 7%, 10%, 30%, 40%), and methanol in order of increasing polarity. The elution was collected in test tubes, each contained approximately 45 mL eluate. The eluate was concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Ten fractions were obtained. TLC (cyclohexane:chloroform:diethylamine (5:4:1), detection by Dragendorff spray) of fraction 5 showed $R_f = 0.15$ (where MLA typically shows $R_f = 0.31$).
TLC (cyclohexane:chloroform:diethylamine (5:4:1), detection by Dragendorff spray) of fraction 5 showed one spot ($R_f = 0.15$) and can be seen under UV light. δ_H ($CDCl_3$) (270

MHz) of the mixture of delavaines A and B (3:2) 11.17 (1H, br s, N*H*CO), 11.08 (1H, br s, N*H*CO), 8.63 (1H, t, J = 8, H-6'), 7.96 (1H, d, J = 7, H-4'), 7.89 (1H, d, J = 8, H-4'), 7.61 (1H, t, J = 7, H-5'), 7.48 (1H, t, J = 7, H-5'), 7.22 (1H, t, J = 7, H-3'), 7.04 (1H, t, J =7, H-3'), 6.90 (1H, s,), 4.09 (2H, m, H-β-18 and H-α-18), 3.85 (1H, s, H-6), 3.58 (1H, dd, J = 5.1 and 5, H-14). 3.39 [3H, s, C(14)OC*H*$_3$], 3.33 [3H, s, C(6)OCH$_3$], 3.32 [3H, s, C(16)OC*H*$_3$], 3.24 [3H, s, C(1)OC*H*$_3$], 3.20 (1H, s, H-16), 3.05 (2H, m, H-9 and H-3"), 2.91-2.92 (3H, m, H-1, H-17, and 1 of NC*H*$_2$CH$_3$), 2.69-2.75 (2H, m, H-α-19 and 1H of NC*H*$_2$CH$_3$), 2.48 (1H, m, H-α-12), 2.41 (1H, m, H-β-12), 2.37 (1H, m, H-β-19), 2.31 (2H, m, H-13 and H-2"), 1.75 (1H, m, H-α-3), 1.70 (1H, m, H-5), 1.67 (1H, m, H-β-3), 1.41 (1H, m, H-5"), and 1.04 (3H, t, J = 7, NCH$_2$C*H*$_3$). $C_{38}H_{54}N_2O_{11}$ requires MW 714, HRMS: m/z of MH^+ $C_{38}H_{55}N_2O_{11}$ requires 715.3800, found 715.3782.

2.4. Isolation of lycaconitine 121 and *N*-succinylanthranoyl lycoctonine 122



121



122

Ground *A. lycoctonum* seeds (80 g) were extracted with hexane (3 x 500 mL) at room temperature. The residue was then extracted with dichloromethane (3 x 500 mL) and ethanol (3 x 500 mL) consecutively in the same process as the extraction by hexane. The hexane extract was concentrated to 300 mL and partitioned with 0.5M sulfuric acid (3 x 200 mL). The acidic layers were combined and then back partitioned with hexane. The combined hexane layers were evaporated in vacuo to give the hexane soluble fraction (not acidic soluble). The pH of the acidic layer was adjusted to pH 7 with 20% sodium carbonate solution and then the solution was extracted with dichloromethane (5 x 100 mL). The combined organic extracts were washed with water (1 x 50 mL), dried ($MgSO_4$), and then evaporated. The dichloromethane extract was concentrated to 300 mL and partitioned with 0.5M sulfuric acid (3 x 200 mL). The acidic layers were combined and then back

partitioned with dichloromethane. The combined dichloromethane layers were evaporated in vacuo to give the dichloromethane soluble fraction (not acidic soluble). The pH of the acidic layer was adjusted to pH 7 with 20% sodium carbonate solution and then the solution was extracted with dichloromethane (5 x 100 mL). The combined organic extracts were washed with water (1 x 50 mL), dried ($MgSO_4$), and then evaporated. The ethanol extract was evaporated to dryness under reduced pressure and the residue was extracted with 0.5M sulfuric acid (3 x 200 mL). The acidic layers were combined and then back partitioned with dichloromethane. The residue after extraction was combined to the organic layer and then evaporated to give the ethanol soluble fraction (not acidic soluble). The pH of the acidic layer was adjusted to pH 7 with 20% sodium carbonate solution and then the solution was extracted with dichloromethane (5 x 100 mL). The combined organic extracts were washed with water (1 x 50 mL), dried ($MgSO_4$), and then evaporated.

Crude dichloromethane extract (180 mg) from *A. lycoctonum* seeds was purified by flash column chromatography on silica gel and the mobile phases were: diethyl ether, methanol in diethyl ether(1%, 2%, 3%, 4%, 6%, 8%, 10%, 30%), and methanol in order of increasing polarity. The elution was collected in fractions, concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Eight fractions were obtained.

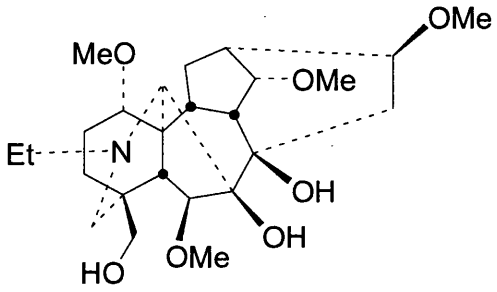
Crude ethanol extract (200 mg) from *A. lycoctonum* seeds was purified by flash column chromatography on silica gel and the mobile phases were: cyclohexane:chloroform: diethylamine (5:4:1), cyclohexane:chloroform:diethylamine:methanol (5:4:1:1), and methanol in order of increasing polarity. The elution was collected in fractions, concentrated and monitored by TLC. The fractions were combined according to the determination of their TLC behaviour. Seven fractions were obtained.

The ^1H NMR spectrum of fraction 3, from cyclohexane:chloroform:diethylamine (5:4:1) of crude ethanol extract, EA-AL-3, showed signals integrating in an 8:1 ratio, which were assigned to lycaconitine **121** (major component) δ_H ($CDCl_3$) (270 MHz) 7.94 (d), 7.60 (t), 7.46 (t), and 7.17 (d) ppm assigned to the anthranilate residue, whilst the minor component *N*-succinylanthranoyl lycoctonine **122** displayed δ_H ($CDCl_3$) (270 MHz) 10.95 (s, carboxylic acid), 8.60 (d), 7.88 (d), 7.24 (s), and 7.01 (t) ppm assigned to its anthranilate residue.

EA-AL-3 was then further purified by analytical HPLC. The condition of this purification is the following: the HPLC column is Phenomenex Luna C18 5μ 150×4.60 mm, the mobile

phase is methanol and formic acid solution (methanol and filtered through a Whatman Nylon membrane prior to use. The acidic solution (0.1% formic acid) was filtered through Milli-Q Plus PF. Methanol was mixed with the 0.1% formic acid in ratio methanol:0.1% formic acid (35:65). The aqueous mobile system was shaken vigorously and degassed on a Decon ultrasonicater for 30 min before use), and detection by UV absorbance at 275 nm. Samples were dissolved in the mobile phase solution and filtered through a 17 mm diameter SYR Filter Nylon 0.45 μm before injection using a 20 μL loop for the analytical column. Peak 4, EA-AL-3-4, and peak 5, EA-AL-3-5, were collected. EA-AL-3-4, HRMS: m/z of MH^+ found 669.3379, MH^+ of lycaconitine **121** $C_{36}H_{49}N_2O_{10}$ requires 669.3387 and EA-AL-3-5, HRMS: m/z of MH^+ found 687.3504, MH^+ of *N*-succinylanthranoyl lycoctonine **122** $C_{36}H_{51}N_2O_{11}$ requires 687.3493.

2.5. Basic hydrolysis of MLA to yield lycoctonine 6



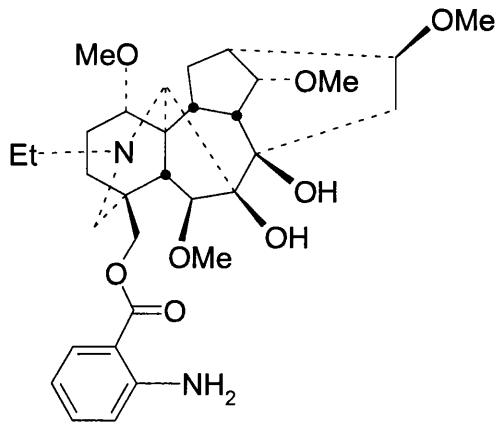
6

To a solution of MLA (0.5 g) in ethanol (10 mL), 5M sodium hydroxide (0.8 mL) was added and stirred 23 h at room temperature. The ethanolic solution was adjusted to pH 7 with 1M hydrochloric acid, concentrated in vacuo and partitioned with dichloromethane (3 x 15 mL). The organic layers were combined, washed with water (1 x 15 mL), brine (1 x 15 mL), dried ($MgSO_4$), and then evaporated in vacuo to give a white solid (91 mg). The aqueous layer was basified with 5M sodium hydroxide to pH 14. The aqueous layer was partitioned with dichloromethane (3 x 15 mL). The organic layers were combined, washed with water (1 x 15 mL), brine (1 x 15 mL), dried ($MgSO_4$), and then evaporated in vacuo to give a white solid which was recrystallized (ethanol) yielding colourless crystals (240 mg, 79%), mp 112-115°C (literature mp 151-153°C, 112-114°C, 95-97.5°C).[3]

TLC (cyclohexane:chloroform:diethylamine 5:4:1, detection by Dragendorff spray and by short wavelength (254 nm) UV light) showed the disappearance of MLA ($R_f = 0.31$) and one homogeneous product ($R_f = 0.18$) and TLC (dichloromethane:methanol: conc. ammonia solution 100:10:1, detection by Dragendorff spray) showed one spot ($R_f = 0.15$). After

detailed comparison with published spectroscopic data (^{1}H and ^{13}C NMR).[3] These crystals were identified as lycoctonine **6** and X-ray data were collected from a single crystal of lycoctonine (hydrate). δ_H ($CDCl_3$) (400 MHz) 4.08 [1H, s, C(8)OH], 3.84 (1H, s, H-α-6), 3.68-3.59 (2H, m, H-14 and H-α-18), 3.44 [3H, s, C(6)OCH_3], 3.41 [3H, s, C(14)OCH_3], 3.35 (1H, d, J = 9), H-β-18), 3.34 [3H, s, C(16)OCH_3], 3.25 [3H, s, C(1)OCH_3], 3.21 (1H, dd, J = 9 and 8, H-16), 3.08-3.05 (1H, m, H-13), 2.96-2.88 (3H, m, H-β-1, H-7, and 1H of NCH_2CH_3), 2.87-2.75 (1H, m, 1H of NCH_2CH_3), 2.63-2.57 (2H, m, H-α-15 and H-α-19), 2.43 (1H, dd, J = 14 and 5, H-α-12), 2.33 (1H, dd, J = 7 and 5, H-10), 2.27 (1H, dd, J = 11 and 2, H-β-19), 2.20-2.01 (2H, m, H-β-2 and H-α-2), 1.94-1.78 (2H, m, H-5 and H-β-12), 1.75 (1H, br s, C(7)OH), 1.69-1.63 (3H, m, H-α-3, H-9, and H-β-15), 1.59-1.47 (2H, m, H-β-3 and C(18)OH), and 1.04 (3H, t, J = 7, NCH_2CH_3); δ_C ($CDCl_3$) (100 MHz) 90.6 (C-6), 88.4 (C-7), 84.2 (C-1), 83.9 (C-14), 82.5 (C-16), 77.4 (C-8), 67.7 (C-18), 64.8 (C-17), 57.9 (C(6)OCH_3), 57.8 (C(14)OCH_3), 56.2 (C(16)OCH_3), 55.8 (C(1)OCH_3), 52.5 (C-19), 51.1 (NCH_2CH_3), 49.5 (C-9), 48.8 (C-11), 46.1 (C-5), 43.2 (C-13), 38.5 (C-4), 38.0 (C-10), 33.5 (C-15), 31.6 (C-3), 28.7 (C-12), 26.1 (C-2), and 14.1 (NCH_2CH_3); $C_{25}H_{41}NO_7$ requires MW 467, HRMS: m/z of MH^+ $C_{25}H_{42}NO_7$ requires 468.2961 found 468.2953.

2.6. Acidic hydrolysis of MLA to yield inuline 7



7

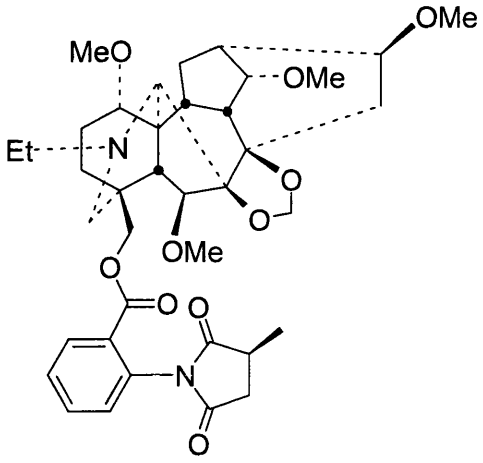
To a solution of MLA (179 mg) in 1,4-dioxane (5 mL), 10% hydrochloric acid (3 mL) was added and stirred for 19 days at room temperature (with TLC monitoring). Solid sodium bicarbonate (5 g) was then added. The solution was partitioned with dichloromethane (3 x 15 mL). The organic layers were combined, washed with water (1 x 15 mL), brine (1 x 15 mL), dried ($MgSO_4$), and then evaporated in vacuo to give a yellow viscous oil. TLC (cyclohexane:chloroform:diethylamine 5:4:1, detection by Dragendorff spray and by short wavelength (254 nm) UV light) showed both MLA and inuline, but the spots were very close in R_f (0.31 and 0.30 respectively) and therefore this was not a practical route to pure inuline.

2.7. Esterification of lycoctonine to yield inuline

To a solution of lycoctonine (250 mg, 0.54 mmol) in anhydrous DMF (4.5 mL) were added isatoic anhydride (176 mg, 1.08 mmol) and 4-(*N*,*N*-dimethylamino)pyridine as catalyst (27 mg, 0.4 eq). The reaction mixture was then heated to 75 ^{0}C and stirred at this temperature for 23 h. TLC (dichloromethane:methanol: conc. ammonia solution 100:10:1) showed that starting material was still present. More isatoic anhydride (51 mg, 0.31 mmol) was added and the mixture stirred at 75 ^{0}C for another 23 h. TLC (dichloromethane:methanol: conc. ammonia solution 100:10:1) showed that starting material was still present. A further portion of isatoic anhydride (51 mg, 0.31 mmol) was added and the mixture stirred at this temperature for another 23 h when TLC (dichloromethane:methanol: conc. ammonia solution 100:10:1) analysis showed that starting material was no longer present. The reaction mixture was cooled to room temperature and partitioned between ethyl acetate (15 mL) and water (15 mL). The aqueous layer was extracted with ethyl acetate (3 x 15 mL). The organic layers were combined and washed with water (15 mL), brine (15 mL), dried ($MgSO_4$) and then evaporated in vacuo to yield a brown oil that was purified over silica gel eluted with 2% methanol in dichloromethane to give a pale yellow oil (37 mg, 12%). This compound was identified as inuline **7**. TLC (dichloromethane:methanol: conc. ammonia solution 100:10:1, detection by Dragendorff spray) showed one spot with $R_f = 0.68$.

TLC (dichloromethane:methanol:ammonia 100:10:1, detection by Dragendorff spray) showed one spot ($R_f = 0.68$) and can be seen under UV light. δ_H ($CDCl_3$) (400 MHz) 8.03 (1H, d, $J = 7$, H-6'), 7.67 (1H, t, $J = 7$, H-4'), 7.53 (1H, t, $J = 7$, H-5'), 7.28 (1H, d, $J = 7$, H-3'), 4.09 (2H, m, H-β-18 and H-α-18), 3.85 (1H, s, H-6), 3.58 (1H, dd, $J = 5.1$ and 5, H-14). 3.39 [3H, s, C(14)OCH_3], 3.33 [3H, s, C(6)OCH_3], 3.32 [3H, s, C(16)OCH_3], 3.24 [3H, s, C(1)OCH_3], 3.20 (1H, s, H-16), 3.05 (2H, m, H-9 and H-3"), 2.91-2.92 (3H, m, H-1, H-17, and 1 of NCH_2CH$_3$), 2.69-2.75 (2H, m, H-α-19 and 1H of NCH_2CH$_3$), 2.48 (1H, m, H-α-12), 2.41 (1H, m, H-β-12), 2.37 (1H, m, H-β-19), 2.31 (2H, m, H-13 and H-2"), 1.75 (1H, m, H-α-3), 1.70 (1H, m, H-5), 1.67 (1H, m, H-β-3), 1.41 (1H, m, H-5"), and 1.04 (3H, t, $J = 7$, NCH$_2$CH_3); $C_{32}H_{46}N_2O_8$ requires MW 586, HRMS: m/z of MH^+ $C_{32}H_{47}N_2O_8$ requires 587.3332, found 587.3299.

2.8. Methylenedioxy acetal formation, conversion of MLA into elatine 3



3

MLA (280 mg) was suspended in formaldehyde diethyl acetal (20 mL) containing a catalytic amount of *p*-toluenesulfonic acid monohydrate (0.5 g). The solution was heated to 80 ^{0}C, then DMSO (3 mL) was added to aid solubilization. The reaction mixture was heated at 80 ^{0}C for 48 h and then cooled to room temperature when toluene (8 mL) was added. The apparatus was rearranged for azeotropic distillation (using a Dean-Stark trap), heated for 2 h, and then cooled to room temperature. The solvents were removed in vacuo, to yield a brown oil that was dissolved in dichloromethane (20 mL) and washed with saturated sodium bicarbonate solution (15 mL). The dichloromethane layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to give a brown oil that was purified by flash column chromatography over silica gel, using 5% methanol in dichloromethane as the eluent which gave a yellow solid (20 mg, 7%). Elatine was obtained higher yield (30%) by using formaldehyde and benzene.[117]

TLC (dichloromethane:methanol:ammonia 100:10:1, detection by Dragendorff spray and can be seen under UV light) of fraction 2 showed two spots (R_f = 0.84 and 0.93) and TLC (cyclohexane:chloroform: diethylamine 5:4:1) of fraction 2 showed two spots (R_f = 0.31 and 0.48). δ_H ($CDCl_3$) (400 MHz) 8.05 (1H, d, *J* = 7, H-6'), 7.67 (1H, td, *J* = 8 and 2, H-4'), 7.53 (1H, td, *J* = 8 and 2, H-5'), 7.27 (1H, dd, *J* = 8 and 1, H-3'), 5.07 (2H, br s, OCH_2O), 4.13-4.00 (2H, m, H-18), 3.43 (3H, s, OMe), 3.35 (3H, s, OMe), 3.33 (3H, s, OMe), 3.26 (3H, s, OMe), 3.30-3.22 (1H, m, H-16), 2.80-2.90 (1H, m, 1H of NCH_2CH_3), 2.76 (1H, d, *J* = 12, H-α-19,), 2.73-2.63 (1H, m, 1H of NCH_2CH_3), 2.60 (1H, dd, *J* = 14 and 4, H-α-12), 2.44 (1H, dd, *J* = 15 and 9, H-α-15), 2.40-2.30 (2H, m, H-β-19, H-13), 1.86 (1H, dd, *J* = 15 and 8, H-β-15), 1.75-1.68 (1H, m, H-β-12), 1.06 (3H, t, *J* = 7, NCH_2CH_3); $C_{38}H_{50}N_2O_{10}$ requires MW 694, HRMS: m/z of MH^+ $C_{38}H_{51}N_2O_{10}$ requires 695.3538, found 695.3532.

2.9. X-Ray crystallography

Aconitine

Commercial aconitine was dissolved in a minimum amount of methanol and the solution was then placed in a desiccator with a beaker of hexane. The solution was allowed to stand for 2 days until crystals formed.

Mesaconitine

Mesaconitine, previously isolated from a commercial sample of *A. napellus* root, was dissolved in a minimum amount of methanol and the solution was then placed in a desiccator with a beaker of hexane. The solution was allowed to stand for 2 days until crystals formed.

Lycoctonine

The white foam from the basic hydrolysis reaction of MLA was dissolved in hot ethanol: hexane (1:1), and allowed to stand and cool until crystals formed.

Delpheline

The solid phase from the crude hexane extract at pH 7.1 and the crude ethanol extract at pH 7.1 were separated by decantation. The material was dissolved in hot ethanol:hexane (1:1), and allowed to stand and cool until crystals formed.

2.10. pK_a measurement

N-Ethylpiperidine (20 mg) was dissolved in CD_3OD:D_2O 3:2 (0.5 mL) in an NMR tube. Chemical shifts of specific resonances (the methylene in the ethyl group and the methyl group) were measured by ^{1}H-NMR and the pH of the solution was measured using an NMR tube pH meter. The pH of the solution was adjusted with NaOD and DCl, and chemical shifts were measured again with the pH meter; a graph of pH vs δ_H was plotted.

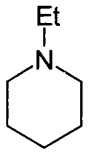
MLA (20 mg) was dissolved in CD_3OD:D_2O 2:1 (0.6 mL). Chemical shifts of a specific resonance (the methyl in the ethyl group attached to nitrogen atom) were measured by ^{1}H-NMR and the pH of the solution was measured using an NMR tube pH meter. The pH of the solution was adjusted with NaOD and DCl, and chemical shifts were measured again with the pH meter; a graph of pH vs δ_H was plotted.

To determine pK_a from the graph of pH vs δ_H, two parallel horizontal lines were drawn along the upper and lower lines of the curve. The former represented the completely unprotonated base and the latter the completely protonated base. A straight line (I) was

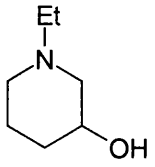
drawn perpendicular to the parallel lines, to measure the distance between them. Another straight line (II) was then drawn from the middle point of I and perpendicular to I, through the curve to the pH axis (y axis). The intercept point on the pH axis is the pK_a.

2.11. Spectroscopy

2.11.1. *N*-Ethylpiperidine



N-ethylpiperidine



N-ethyl-3-hydroxypiperidine

The ^{1}H-, ^{13}C- and DEPT NMR spectra of *N*-ethylpiperidine are as expected: δ_H ($CDCl_3$) (270 MHz) 2.08 (6H, q, H_2-1, H_2-6 and NCH_2CH_3), 1.32-1.27 (4H, m, H_2-3 and H_2-5), 1.15-1.14 (2H, m, H_2-4) and 0.77 (3H, t, NCH_2CH_3); δ_C ($CDCl_3$) (100 MHz): 54.2 ppm (NCH_2CH_3), 53.1 (C-2 and C-6), 26.1 (C-3 and C-5), 24.7 (C-4) and 12.2 (NCH_2CH_3). Only five peaks appear in the ^{13}C-NMR spectrum as this molecule has an axis of symmetry.

2.11.2. *N*-Ethyl-3-hydroxypiperidine

N-Ethyl-3-hydroxypiperidine is found embedded in many norditerpenoid alkaloids. In order to aid the spectroscopic assignments it was analysed as a model compound. Unlike *N*-ethylpiperidine, for *N*-ethyl-3-hydroxypiperidine the rate of conformation change of axial and equatorial protons is slow enough to show their different environments. The assigned peaks: δ_H ($CDCl_3$) (270 MHz) 0.79 (NCH_2CH_3), 3.44 (H-3) and 4.74 (C(3)O*H*); δ_C ($CDCl_3$) (100 MHz) 66.3 (C-3), 60.7 (C-2), 33.3 (C-4), 23.1 (C-5) and 11.9 (NCH_2CH_3). The peaks at 52.5 and 53.0 ppm cannot be assigned unambiguously between NCH_2CH_3 and C-6.

2.12. Membrane preparation and [^{125}I] α-bungarotoxin binding assays

These experiments were carried out in collaborative studies with Prof S. Wonnacott and Mr P. Livingstone, Univeristy of Bath. Male Sprague-Dawley rats (180-220 g) were sacrificed by cervical dislocation, brains were removed and homogenised in 0.32 M sucrose buffer (pH 7.4, 4 °C). Particulate membranes were purified by a series of centrifugations and incubated in phosphate buffer containing 1 nM [^{125}I]-α-bungarotoxin with or without the competing ligand of interest. After filtration (Brandel Cell Harvester), specifically bound radioligand was determined.[103, 104]

3. Results and discussion

3.1. Extraction

3.1.1. Methods

It was important to optimize the extraction of the seeds of Delphinium cv Pacific Giant by minimising the amounts of time and solvents whilst producing consistently a maximum yield of crude alkaloid. The seeds of *Delphinium* are considered to be the most alkaloid rich parts of the plant. The extraction of the seeds can yield up to 2% alkaloidal material.[18] However, the usual value is 0.2-0.7% of the total dry weight of the plant material.[118, 119]

In the traditional extraction process, extraction was performed by repeated maceration with agitation and percolation. The extracts were then taken through an acid/base cycle which involved a number of involved filtration, separation, and partitioning steps as well as evaporations under reduced pressure which were inclined to bump and which would present many difficulties in larger scale experiments. Even though this method afforded a high yield of crude alkaloidal material and used only modest volumes of solvents, the procedure was considered to be laborious and problematical. The problems from the scaling-up of this procedure might be minimised with effective stirring and control of the temperature. The use of a Soxhlet continuous extractor aimed to both optimize the extraction and avoid the practical problems of removing large volumes of solvents encountered in the traditional extraction process.

The laboratory apparatus for the Soxhlet continuous hot extraction is the Soxhlet extractor, in which the solvent in the flask is heated to boiling point and the vapour condensed in the reflux condenser. The hot liquid drips onto the plant sample, which is contained in a porous thimble. When the liquid in the extraction chamber reaches the top of the siphon tube, it flows back into the heated flask, taking with it any dissolved plant material. The plant material is thus repeatedly extracted and the soluble components are concentrated in the flask. The disadvantage with this method is that the extracted material is subjected to continuous heating and it cannot therefore be used if the material is thermolabile. Using a low boiling-point solvent could avoid this problem. However, trace impurities such as plasticisers can also be a problem of extraction.

Another problem anticipated in the traditional extraction method is the practical problem of separation of emulsions. The defatting process prior to the extraction is introduced to minimise that problem and optimize the extraction. Fats and/or oils, such as long-chain saturated or unsaturated triglycerides, which often occur in seeds, are usually insoluble in alcohol and water and soluble in ether and hexane. In our studies, we found hexane to be useful for the removal of these lipids. The clarity of the extracts was used as an indication of effective defatting. However, in this study significant amounts of alkaloid were also extracted by hexane.

To separate alkaloids from the crude extract, liquid/liquid extraction is a commonly used technique requiring two immiscible solvents and involving partition of the solute molecules between the two phases produced. The amount of solute in each phase will depend upon the relative solubility in each solvent which, in turn, is related to their polarity. It is measured by the partition coefficient which, for any system, is a constant provided that neither phase becomes saturated with solute molecules.

$$\text{Partition coefficient} = \frac{\text{mole fraction of solute in phase 1}}{\text{mole fraction of solute in phase 2}}$$

The success of this method depends upon the selectivity of the solvents for the required compound. A refinement of this technique is the liquid/liquid partition method known as counter-current distribution. When a solute is being extracted from an aqueous phase using an organic solvent, a better recovery will be obtained by using two equal volumes of solvent than the recovery that would be obtained using all the solvent in one large volume.

Alkaloids, as weak bases, form salts with strong acid (sulfuric acid), so they can be separated from non-basic compounds. It is possible to affect the degree of separation of by manipulation of pH of the aqueous phase in a liquid/liquid partition system. It is a general rule that salts, being ionic, are soluble in polar and insoluble in non-polar solvents, and thus alkaloids will dissolve in aqueous acids as their salts. By making the aqueous phase basic the situation is reversed, the alkaloid salts being converted into the free bases which have greater affinity for the organic phase. Usually the pH is adjusted to a value roughly between the pK_a constants of the compounds to be separated. Weak bases like ammonia or sodium bicarbonate ($NaHCO_3$) are used for moderately basic pH values while stronger bases like potassium carbonate (K_2CO_3) or sodium hydroxide (NaOH) are used for strongly alkaline conditions. This mechanism allows specific isolation of alkaloids from other, non-basic, materials and it can be used to separate very weak basic alkaloids from very strong basic

alkaloids as long as the difference of pK_a is large enough. However, it is not possible to separate chemically similar acids or bases using this simple method. In this study, MLA **4** was found in hexane soluble alkaloidal extract, dichloromethane soluble alkaloidal extract, and ethanol soluble alkaloidal extract and at every pH used in the acid/base cycle but at varying concentrations. Furthermore, TLC chromatograms showed that the patterns of the hexane soluble alkaloidal extract at three different pH fractions were similar, but the patterns of the dichloromethane soluble alkaloidal extract and the ethanol soluble alkaloidal extract were different, especially the spots with higher polarity than MLA **4**.

Seeds of *A. lycoctonum* were extracted by repeated cold extraction at room temperature allowing three days maceration with each change of solvent. The disadvantages of this method are that a large amount of solvent is required and it is more time consuming than Soxhlet continuous hot extraction. However, the advantage with this method is that the thermolabile compounds are extracted possibly without decomposition.

3.1.2. Choice of solvent

It is possible to use a solvent that will dissolve out the alkaloids, or one that will remove the other compounds to leave higher concentration of the alkaloids in the plant samples. The most important factor influencing the solubility of material is the polarity of the solvent and the solute molecules. The extraction effectiveness is determined by the affinity of molecules of the solvent for molecules of the solute over their affinity for each other. For the good extraction, the solvent molecules must have a high attraction for the solute molecules. It is therefore necessary to consider the possible forces that may occur between solvent molecules and the way in which solute molecules can interfere with them.

In non-polar solvents the only force present between molecules is that of dispersion. Where molecules of the solvent have a permanent dipole, dipole-dipole force will exist, causing the solvent molecules to be more strongly attracted to each other and to resist the introduction of a non-polar molecule. Induced interactions may occur when a molecule with a permanent dipole approaches a non-polar molecule possessing mobile electrons. This produces an induced temporary dipole which will assist in the dissolution of such a molecule. Finally, the formation of hydrogen bonds must be considered. Although hydrogen is monovalent, it can form a weak secondary bond with electron rich atoms.

It is often found that compounds have features that confer both polarity and non-polarity to the same molecule. The ground seeds had many components varied in polarity and other properties so it is impossible to separate a single pure alkaloid by using one solvent.

The choice of solvent for extraction of the crude bases from the powdered seeds is significantly important. A variety of extraction solvents are widely accepted, including dichloromethane, but alcoholic solvents are generally used for many plant constituents.

By concerning of polarity, alkaloids have different polarities depending on the structure and the substituents so they dissolve in different solvent by the rule 'like dissolves like'. In our studies, three solvent systems were used to extract the crude alkaloidal material. The first system is hexane, dichloromethane, and ethanol in order of increasing polarity. The second is hexane and ethanol and the last is hexane and chloroform. Hexane is considered to be suitable solvent not only for defatting but also for extracting the crude alkaloidal material which is non-polar and/or low polar.

In a Soxhlet experiment, after defatting with hexane, dichloromethane followed by ethanol were used as extraction solvents. This extraction process showed the band of MLA **4** by TLC (cyclohexane:chloroform:diethylamine 5:4:1, detection by Dragendorff spray) for three extracts and obtained 2.3 % of overall yield of crude alkaloid (hexane soluble alkaloidal extract 0.8%, dichloromethane soluble alkaloidal extract 0.4%, and ethanol soluble alkaloidal extract 1.1%).

Two other Soxhlet regimes were experimented because they were quicker methods to obtain specific alkaloid, MLA **4**. The ground seeds were defatted by hexane and then extracted by ethanol. This gave the band of MLA **4** by TLC (cyclohexane:chloroform:diethylamine 5:4:1, detection by Dragendorff spray) for both extracts and obtained 4.1% of overall yield of crude alkaloid (hexane soluble alkaloidal extract 2.7% and ethanol soluble alkaloidal extract 1.4%). The apparent overall yield of this process was high because of the high yield of hexane soluble alkaloidal extract, maybe due to the presence of residual fixed oil from emulsion formation during the partition process.

In the other extraction process, the ground seeds were defatted by hexane and then extracted by chloroform, showed the band of MLA **4** by TLC (cyclohexane:chloroform:diethylamine 5:4:1, detection by Dragendorff spray) for both extracts and obtained 0.43% of overall yield (hexane soluble alkaloidal extract 0.16% and chloroform soluble alkaloidal extract 0.27%).

By comparison to the experiment of Coates and coworkers, the overall yield of this experiment is less than their experiment (1.22%).[120]

From the results of the extraction, the generally used solvents are hexane (for defatting and extraction), dichloromethane, and methanol. However, the alkaloidal material from the extraction of the seeds of *Delphinium* cv Pacific Giant collected in 1989 with this solvent system did not show the band of MLA by TLC (cyclohexane:chloroform:diethylamine 5:4:1, detection by Dragendorff spray). Thus, the seeds collected in 1993 were extracted with the same process, obtaining the alkaloidal material that showed the band of MLA and other bands by TLC (cyclohexane:chloroform:diethylamine 5:4:1, detection by Dragendorff spray).

The essential oils are generally more volatile than the alkaloids, thus their content decreased over a period of time quicker that that of alkaloids. However, Browinska-Szmalowa presented that the content of alkaloids from *Atropa belladona*, *Datura stramoninum*, *Datura stramoninum* var. *inermis*, *Aconitum napellus*, and *Aconitum lycoctonum* decreased in decaying plants.[121] Furthermore, Jermstad showed that the alkaloid content of *Aconitum septentrionale* (synomymous with *A. lycoctonum*) decreased by 11.2% over a period of ten years. The disappearance of MLA may due to the time and condition of storage.[122]

3.2. Purification

3.2.1. Detection by TLC and HPLC

TLC can be used as an aid in the identification of compounds. This identification is based on the distance a compound elutes in a particular chromatographic system, the R_f value. This is defined as the ratio of the distance moved by the compound over the distance moved by the solvent front. In theory, the R_f of a compound is constant provided that all the chromatographic conditions are kept constant. This is, however, very difficult to achieve in practice as there are many factors that affect R_f. The prime importance is the amount of moisture adsorbed on the plate. The presence of moisture produces a deactivated plate. Therefore, the more water adsorbed on to a plate, the greater will be the R_f value of any particular compound. It is extremely difficult to measure or control this moisture content. Tank saturation is also important. Where the tank is not saturated with eluent vapour the R_f of the compound being examined appears higher than in a saturated system. This is because the solvent evaporates as it travels up the plate, thus producing a lower solvent front than

would be produced in a saturated tank. When the R_f value is calculated, it will reflect this difference. Temperature is not particularly important except where the tank is unsaturated, and in most cases the sample loading is also unimportant.

The R_f value will be useful for identity when a known compound is subjected to chromatography at the same time and in the same system as the unknown. As both materials are run at the same time, they are both subjected to the same variables. However, it must be point out that R_f value cannot be taken as proof of identity. Materials that have the same R_f in a particular system will co-chromatograph (that is they will not separate in that system) and consequently the appearance of a single spot is not absolute proof of purity.

Most alkaloids are detected by Mayer's reagent (potassiomercuric iodide solution); by Wagner's reagent (solution of iodine in potassium iodide); by solution of tannic acid; by Hager's reagent (a saturated solution of picric acid); or by Dragendorff's reagent (solution of potassium bismuth iodide). The results are of various colours: cream (Mayer's), yellow (Hager's), reddish-brown (Wagner's and Dragendorff's). Dragendorff's reagent was commonly used to detect alkaloids and there are many modified Dragendorff's reagents.

In our studies, four reagent sprays were used to detect the alkaloids: iodine vapour, vanillin solution, sulfuric acid solution, and Dragendorff's reagent. The results showed the iodine vapour gave brown spots, but they disappeared quickly. The vanillin solution presented different colours depending on the type of compounds. The sulfuric acid solution obtained black spots after charring, but it is not convenient. Both vanillin solution and sulfuric acid solution are not specific for detecting alkaloids. Dragendroff reagent spray yielded the red-brown spots and the spots stayed longer time. This spray is more specific for detecting alkaloids. Even though Dragendorff reagent spray is suitable for detecting the alkaloids isolated from the seeds of Delphinium cv Pacific Giant and *A. lycoctonum*, other problem of detecting alkaloids still exist. This frequently used spray is not sensitive for dilute concentrations of alkaloids, even sometimes for concentrated alkaloid solutions! However, the alkaloid caffeine cannot be detected by Dragendorff reagent spray at all.

The most widely used method of detection in HPLC is UV absorption, being sensitive, reproducible, and easy to operate. With sensitivities of 0.001 absorbance unit, full scale deflection, and noise levels typically 1%, it is possible to detect 1 ng of solute.[123] The wide linear dynamic range (10^4) of these detectors makes it possible to measure both trace and major components on the same chromatogram. Ideally the detector should have a usable

sensitivity of better that 0.1 μg of sample in 1 cm^3 of mobile phase. However, the sensitivity for suitable trace solutes depends entirely on UV characteristics of the compound. According to Cookson and Trevett,[124] MLA showed a UV absorption maximum at 273 nm with a molar extinction coefficient (ε) 3,500 accordingly a detection wavelength of 275 nm was used in this work. However, this depends on the fact that MLA possesses the methylsuccinimidoanthranylate chromophore. Other alkaloids such as delpheline do not possess this chromophore and cannot be detected at this wavelength. This is a problem with many alkaloids not possessing any chromophores. LC-MS and LC-MS-MS techniques have been used to detect alkaloids regardless of the possessing of the chromophore or UV absorbance.[125, 126]

3.2.2. Vacuum liquid chromatography (VLC)

Coll and co-workers first mentioned vacuum liquid chromatography for the separation of diterpenes, but experimental details were not reported.[127, 128] This technique was elaborately described for the separation of a standard dye mixture, including its effectiveness.[129]

The chromatographic separation of norditerpenoid alkaloids of *Delphinium* and *Aconitum* have been studied.[130] Most of the isolation methods are expensive, high time consuming, or useful only for small-scale separations. VLC is useful for the separation of crude extracts of natural products. In our studies, the apparatus was modified (see Experimental) and a vacuum pump was used instead of a water aspirator. This experiment gave a poor separation with each fraction containing more than one compound, TLC (cyclohexane: chloroform:diethylamine 5:4:1, detection by Dragendorff spray) showed at least three spots.

3.2.3. Flash column chromatography

This technique is rapid for preparative separation with moderate resolution.[111] There are numerous forms of chromatography but they all have in common the fact that separation is due to the preferential retention of solute from a mobile phase at a non-mobile interface. The interface may commonly be liquid/liquid (similar to the counter-current separation), liquid/solid, and gas/liquid. There are two fundamental physico-chemical forces that may be involved in chromatography. The first of these is partition. The important difference between partition in simple separations and in chromatography is that in chromatography one of the immiscible phases is mobile and the surface area of the non-mobile phase is far

greater that could be obtained for the same volume in a separating funnel. This means that the equilibrium of solute between the two phases cannot be established.

Silica gel and alumina can also be used to separate mixture due to adsorption forces, solute molecules being actively adsorbed onto the stationary phase from the mobile phase. This process, which is liquid/solid separation, is known as adsorption chromatography and most chromatographic processes are a mixture of both partition and adsorption chromatography.

In the adsorption chromatography the competition between the solute and solvent molecules for the active sites is a dynamic one and different solute molecules will have varying degree of success in this competition. Polarity is of prime importance when considering the choice of solvent for the extraction process and so it is not surprising to find that it is important in the selection of chromatographic systems. It is possible to list solvents in order to their ability to retain solute molecules in the mobile phase of a chromatographic system, such a list being termed an elutropic series. This series closely reflect the polarity of the individual solvents. In adsorption chromatography the choice of solvent (eluent) depends upon the competition between the solvent and solute molecules for the active sites of the adsorbent. Where the solvent is too strong for the solute, the solute will remain entirely in the mobile phase. If the eluent is too weak, the solute molecules will remain bound to the adsorbent. Neither case is desirable as in both instances the equilibrium is all in one direction and slight differences between similar solute molecules cannot be seen. Where an intermediate solvent is used related compounds can be separated as the individual compound molecules have an opportunity to demonstrate slight differences in their ability to compete with the eluent molecules

In order to obtain maximum efficiency the column must be evenly packed. While column chromatography using gravity feed of solvent is restricted to packing particles more than 150 μm (in order to obtain acceptable flow rates), the column must be packed as uniformly as possible to minimise distortion of the chromatographic boundaries. Channelling is usually caused by the inclusion of air bubbles during packing. To prevent these effects, the packing material should be slurried with the solvent and poured as a thin stream into the column, which should be about one third full of solvent. If the adsorbent is allowed to settle gradually, reasonably homogenous packing will result. On no account should any part of the column be allowed to run dry, during packing or during a separation.
The columns were wet packed, that is the adsorbent was added to the mobile phase already in the column or slurry packed where the adsorbent and eluent were slurried together and

poured into the chromatography column. The choice of method depends to a large extent on the density of the adsorbent. Alumina is too heavy to form a stable suspension in most eluents and cannot be slurry packed. The material to be separated was applied to the top of the column in as concentrated a form as possible (the concentrated solution in minimum volume or the solid phase dispersed on an appropriate amount of Celite). The mobile phase was allowed to run through the adsorbent under the force of gravity and be replaced by more eluent from a reservoir. The components of the mixture were separated according to their retention by the adsorbent, and obviously a solvent of suitable elutropic power must be used.

Frequently, in the analysis of complex plant mixtures the range of polarities of compounds is so great that no one solvent is suitable. In this case, a technique known as gradient elution is employed. In this, instead of continuing to pass one solvent, or a single solvent mixture, the nature of the solvent is changed throughout the separation. Thus, a column set up in diethyl ether might be run successively in 1% methanol in diethyl ether, 2% methanol in diethyl ether, 5% methanol in diethyl ether, 10% methanol in diethyl ether, 20% methanol in diethyl ether, and 50% methanol in diethyl ether. Non-polar compounds are eluted at the beginning and progressively more polar compounds emerge as the polarity of the eluent increases.

Monitoring the progress of a column separation will be simple if the material to be separated was coloured. Frequently, however, colourless compounds are separated by column chromatography so fractions of eluent are collected, concentrated and monitored by analytical TLC which can be observed under UV light, short and/or long wavelength, by spray reagents.

The main application of this type of chromatography is in large scale preparative work as it is capable of handling up to 100 g of material without undue difficulty. Consequently it may be used to effect a crude separation of material at the beginning of a purification procedure or at the end of a commercial isolation when purity is required for the finished product.

A term which is used in connection with adsorbents is activity. This can relate to the specific surface of the solid or strength of adsorption. This is usually the sense referred to in chromatography and is the one that will be employed in our studies.

In our studies, silica gel and alumina (basic, neutral, and acidic) were used as the adsorbents. However, the activity of the active sites of the adsorbent is varied depending upon the type

of the adsorbent. Silica gel is a purified silicon dioxide (SiO_2). The active sites on the surface consist of silanol groups which are spaced approximately 5 Å apart. The surface interacting with polar solutes chiefly by mean of H bonding and due to the acidic nature of the surface basic substances is held particularly strongly. The principal problem associated with silica (and adsorbents in general) is the tendency to cause peak tailing. An additional hazard is that irreversible adsorption may take place on columns and for this reason complete recovery of the adsorbates is not always achieved. Isomerisation and other reactions of various compounds such as terpenes and sterols have been reported to occur on silica gel.

The alumina surface is capable of exhibiting different types of solute-solvent interaction. This may be attributed to, first, the positive fields surrounding Al^{3+}, which allow interaction with easily polarisable molecules. Second, basic sites were allowed interaction with proton donors. Alumina is less widely used than silica due to its propensity to catalyse reactions with base-labile molecules and to cause rearrangements and even ring expansion in unsaturated and alicyclic compounds.[131]

Solvent is an important part in the adsorption process and competes with the sample molecules for active sites on the adsorbent. Thus, the stronger the binding of solvent molecules, the greater the amount of time the solute molecules spend in the mobile phase, and hence the faster they are eluted. Retention is therefore not so much influenced by sample solubility in the eluent as by the strength of solvent adsorption. It is advisable for a given application to choose an initial solvent of indifferent eluting power, so that stronger solvent system can be tried subsequently. In our studies, many mobile phase systems are used to obtain suitably chromatographic performances. Most of mobile phase system is gradient elution. This technique involves the use of a continuously changing eluting solvent. The effect of this gradient is to elute successively the more strongly adsorbed substances and at the same time to reduce tailing. This means that the chromatographic bands will tend to be more concentrated and thus occupy less of the column.

3.2.4. High performance liquid chromatography (HPLC)

Reversed phase HPLC (RP-HPLC) consists of a non-polar stationary phase and an aqueous, moderately polar mobile phase. In our studies, the stationary phase is a silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group with 18 carbon atoms. The retention time is therefore longer for molecules which are more non-polar in nature, allowing polar molecules to elute more readily. Retention time is increased by the addition

of polar solvent to the mobile phase and decreased by the addition of more hydrophobic solvent. Reversed phase chromatography (RPC) is so commonly used that it is not uncommon for it to be incorrectly referred to as "HPLC" without further specification. The pharmaceutical industry regularly employs RPC to qualify drugs before their release.

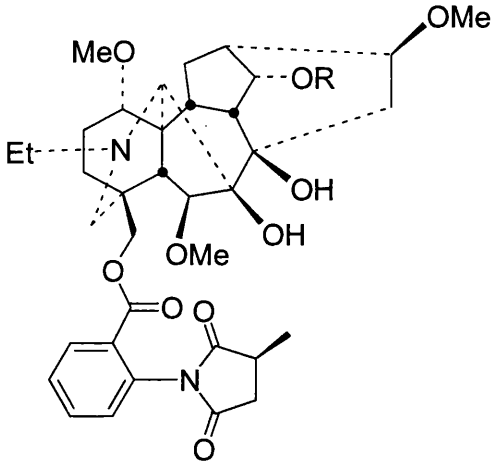
RPC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent. This solvophobic effect is dominated by the force of water for "cavity-reduction" around the analyte and the C18-chain versus the complex of both. The energy released in this process is proportional to the surface tension of the eluent and to the hydrophobic surface of the analyte and the ligand respectively. The retention can be decreased by adding less-polar solvent (methanol and acetonitrile commonly used) into the mobile phase to reduce the surface tension of water.

Structural properties of the analyte molecule play an important role in its retention characteristics. In general, an analyte with a larger hydrophobic surface area (C-H, C-C, and generally non-polar atomic bonds, such as S-S and others) results in a longer retention time because it increases the molecule's non-polar surface area, which is non-interacting with the water structure. On the other hand, polar groups, such as -OH, $-NH_2$, COO^- or $-NH^{3+}$ are reducing retention as they are well integrated into water. Very large molecules, however, can result in an incomplete interaction between the large analyte surface and the ligands alkyl chains and can have problems entering the pores of the stationary phase. Retention time increases with the hydrophobic surface area.

Reversed phase columns are quite difficult to damage compared with normal silica columns, however, many reversed phase columns consist of alkyl derivatized silica particles and should never be used with aqueous bases as these will destroy the underlying silica backbone. They can be used with aqueous acid but the column should not be exposed to the acid for too long, as it can corrode the metal parts of the HPLC equipment. The metal content of HPLC columns must be kept low if the best possible ability to separate substances is to be retained.

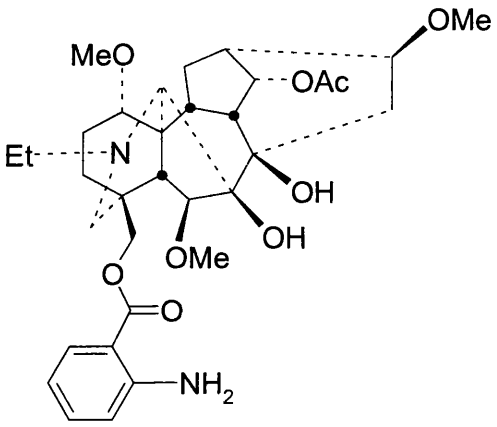
3.3. Norditerpenoid alkaloids from Delphinium cv Pacific Giant

Delphinium cv Pacific Giant is an ornamental hybrid garden cultivar. This cultivar is hybridised or selected mainly from *D. elatum*. From this species, many constituents have been investigated. From the seed oil of *Delphinium*, C20 fatty acids were obtained, including low levels of 18:3n-3 fatty acids.[132] However, our studies focus on alkaloidal constituents and many alkaloids have been investigated. The following norditerpenoid alkaloids have been found in the seeds of *D. elatum*: 14-deacetylnudicauline **123**[133], andersonidine **124**[134], blacknine **125**[135], blacknidine **126**[135], delcorine **127**[134], delectinine **128**[136], delelatine **2**[137], delpheline **1**[133, 138], deltaline **5**[133], eladine **129**[133, 134], elanine **130**[136], elasine **131**[133], elatine **3**[133], isodelpheline **132**[133], lycoctonine **6**[133], MLA **4**[133], nudicauline **133**[133], pacidine **134**[134], pacifidine **135**[134], pacifiline **136**[134], pacifinine **137**[134], paciline **138**[138], pacinine **118**[136, 138], and yunadelphinine **139**[134].



123 **R = H**

133 **R = Ac**



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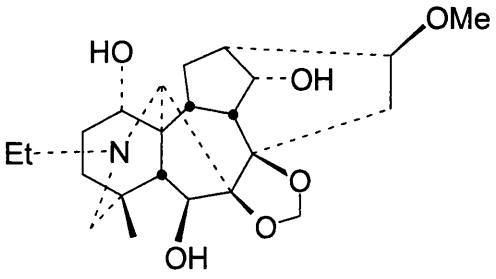

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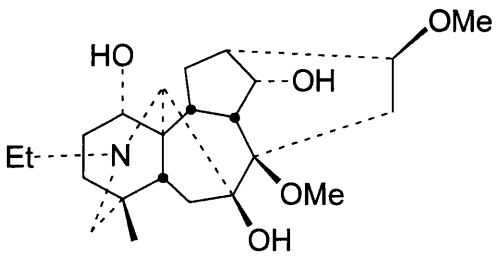

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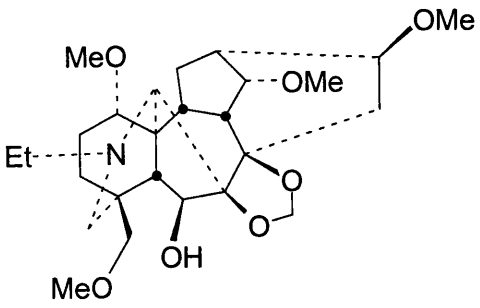

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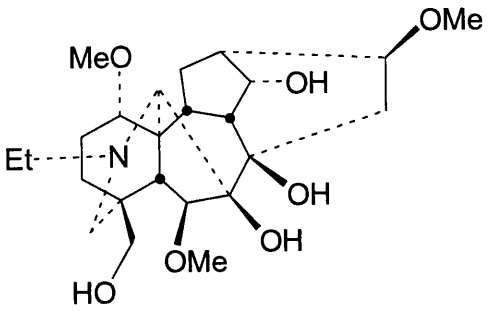

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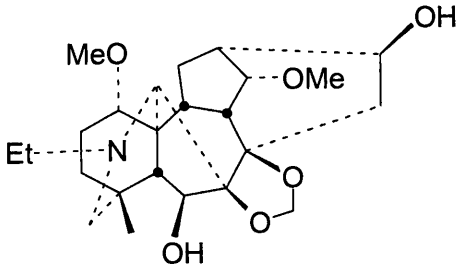

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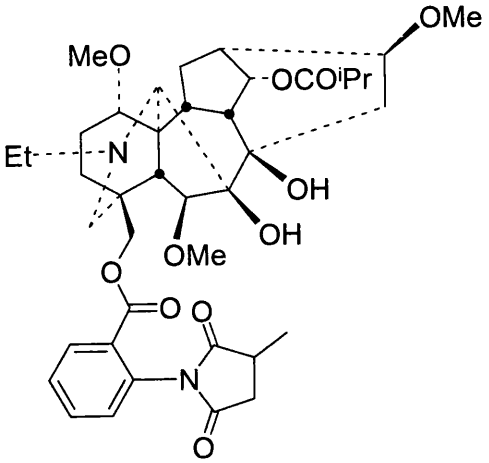

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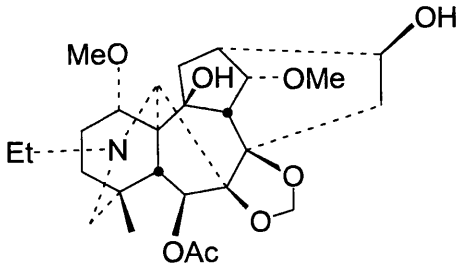

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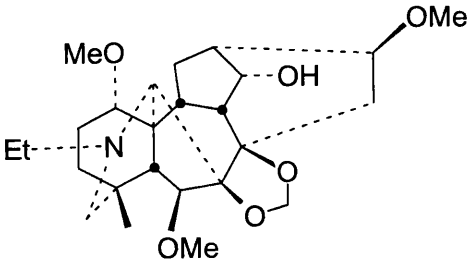

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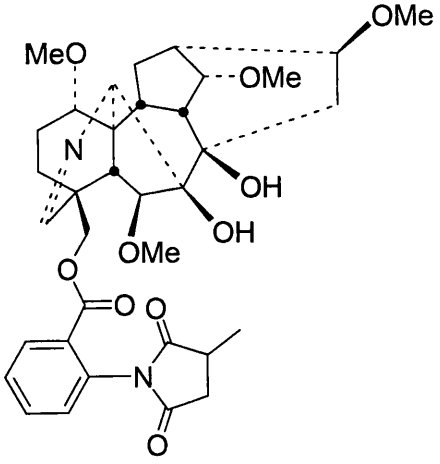

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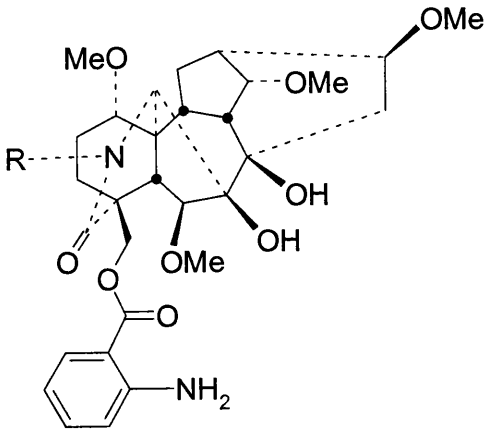
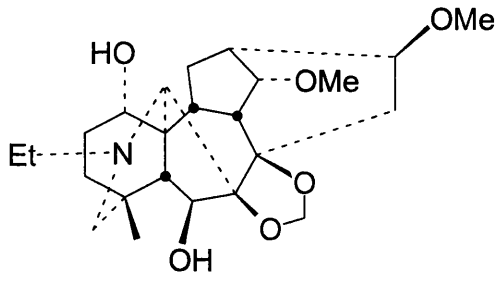


136 **R = Et**

137 **R = H**



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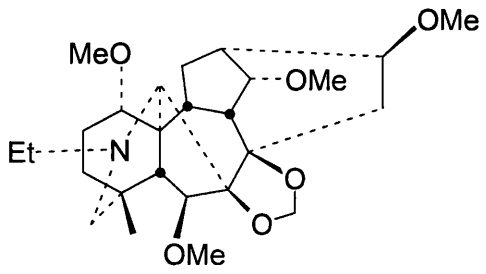

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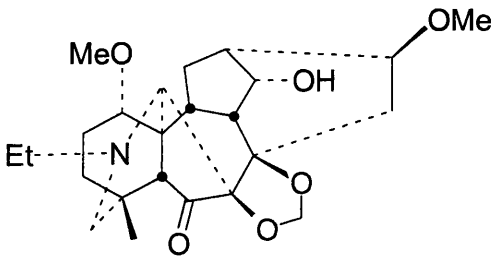
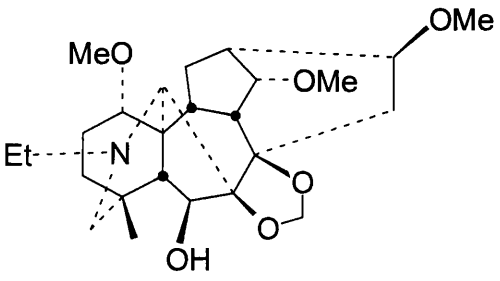


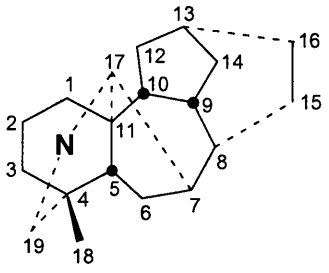
139

In our studies, four known compounds, delpheline **1**, methyllycaconitine **4**, pacinine **118**, and delavaine A and B (**119**,**120**) were isolated from the seeds of Delphinium cv Pacific Giant.

Hanuman and Katz, and Pelletier and co-workers have collated ^{13}C and ^{1}H NMR data, and by averaging over a large number of compounds having similar structural features, the general ranges in chemical shifts for the various skeletal and functional groups are available and were useful in establishing the structures of these norditerpenoid alkaloids.[3, 4]

3.3.1 Delpheline 1





1

Delpheline was dissolved in $CDCl_3$ and its ^{1}H-NMR spectrum recorded at 400 MHz. Assignments were made by 1D, 2D ^{1}H, ^{13}C, DEPT, HMQC, and HMBC techniques. Figure 3.1 shows the ^{1}H NMR spectrum (400 MHz), Figure 3.2 is the ^{13}C NMR spectrum, Figure 3.3 is the DEPT spectrum (100 MHz), Figure 3.4 is the HMQC spectrum and the HMBC

spectrum is the Figure 3.5. Delpheline which has been reported from Delphinium cv Pacific Giant, *D. elatum*, *D. barbeyi*, *D. occidentale* and *D. ternatum*.[139] The DEPT showed the presence of four quaternary, nine methine, seven methylene, and five methyl carbons and the ^{1}H NMR spectra (Figure 3.1) presented three methoxy signals (δ3.26, 3.35 and 3.43 ppm) and peaks at δ5.05 and 5.13 ppm from a methylenedioxy group between C-7 and C-8, and the mass spectrum are broadly in agreement with the data reported for the norditerpenoid alkaloid delpheline. However, there are some inconsistencies in the ^{1}H NMR assignments reported in the literature of delpheline[138, 140], so, in order to be confident of the substitution sites, it was necessary to carry out a more detailed examination of the NMR data.

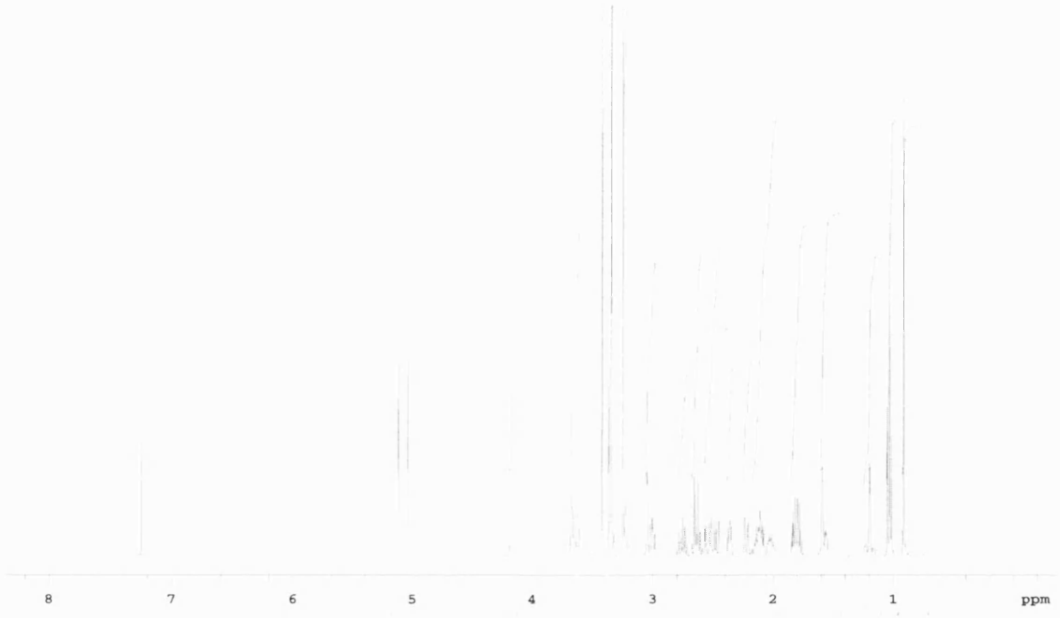


Figure 3.1 ^{1}H-NMR spectrum of delpheline

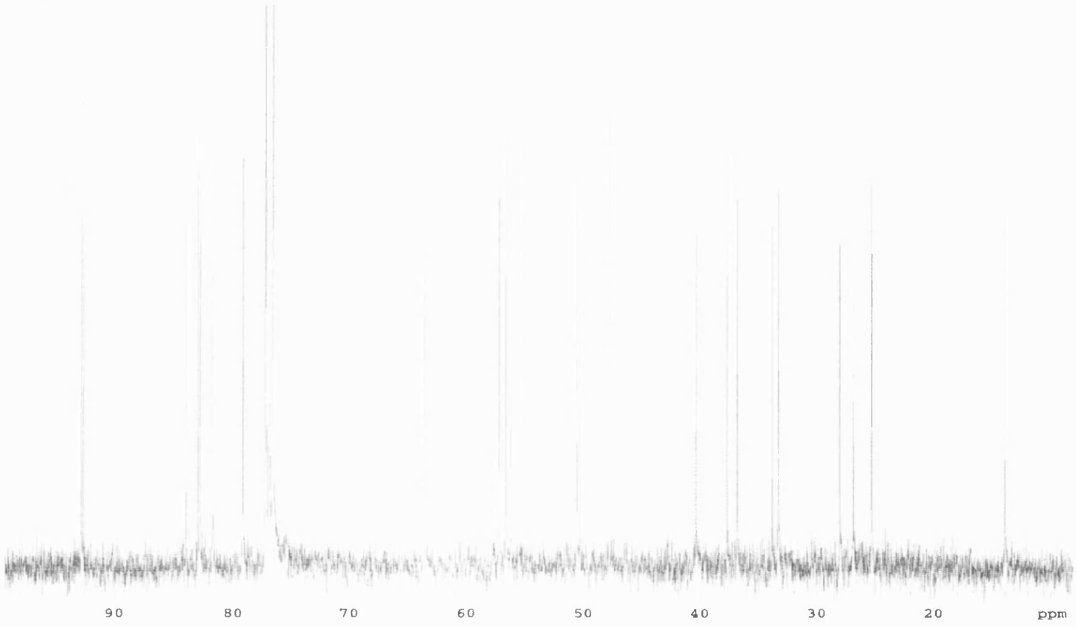


Figure 3.2 ^{13}C-NMR spectrum of delpheline

Spectral data for delpheline were summarised in Table 1 and allowed us to confidently assign C(1)OCH_3 by observing three bond interactions between C-1, C(1)OCH_3, and H-1 in HMQC and HMBC NMR techniques (Figures 3.4 and 3.5). This was possible because the assignment of H-β-1 is undisputed due to the coupling observed to other ring A protons in the HMBC spectrum (Figure 3.5). Moreover, C(16)-β-OCH_3 was assigned, based on the assignment of H-α-16 (δ3.25-3.19 ppm) by HMBC technique with other ring D protons (H-α-15 and H-β-15) (Figure 3.5). The HMQC and HMBC techniques showed that H-16 was coupled to 56.3 ppm of C(16)OCH_3 and the carbon at 81.8 ppm of C-16 interacted with δ3.35 ppm of C(16)OCH_3.

A crosspeak between H-β-14 and C(16)-β-OCH_3 is observed in the HMBC spectrum (Figure 3.5). Subsequently, ^{1}H NMR (Figure 3.2) and ^{13}C NMR signals (Figure 3.3) for each of the three *O*-methyl ethers at positions 14, 1, and 16 in delpheline can be unambiguously assigned at: δ3.26, δ3.35, and δ3.43 ppm, 55.5, 56.3, and 57.8 ppm respectively. Our spectral data for the C-1 and C-16 methoxy positions (Figures 3.3 and 3.4) are in agreement with the assignments given in the literature by Joshi and co-workers.[140] An unfortunate transposition in their table of results appears to have arisen.[140] We can also agree with the important C-1 α-substituent reassignment made by Pelletier and co-workers on observing an interaction between H-β-1 and H-β-10 in the NOESY spectrum and that between C(1)-OCH_3 and H-α-12.[141]

Detailed comparison of ^{13}C NMR spectrum (Figure 3.3) with those published for isodelpheline, the C-14-OH, C-16-OCH_3 regioisomer[139, 142] leads us to believe that the isolated base is delpheline and not isodelpheline. The data for isodelpheline shows 74.1 (C-14), 83.5 (C-1), 89.2 (C-6), and 93.7 ppm (OCH_2O),[142, 143] whereas for delpheline we found: 83.0 (C-14), 82.7 (C-1), 72.9 (C-6) and 92.9 ppm (OCH_2O), indicating that a downfield shift of approximately 10 ppm is observed for a methoxy substituted carbon compared to a hydroxyl substituted carbon.

A sample of delpheline was recrystallized (ethanol:hexane) and biological activity measurements were carried out (in collaborative studies) and the data compared with four structurally related norditerpenoid alkaloids which we purified from Delphinium cv Pacific Giant (*vide infra*).

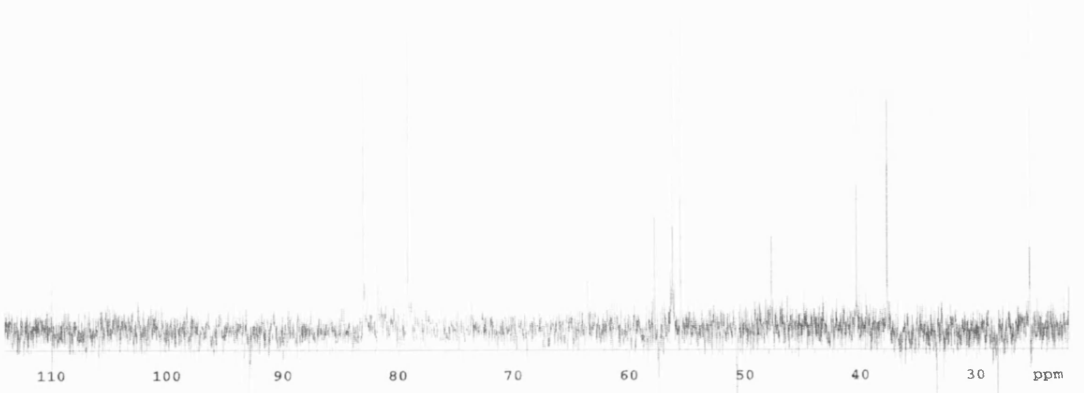


Figure 3.3 DEPT spectrum of delpheline

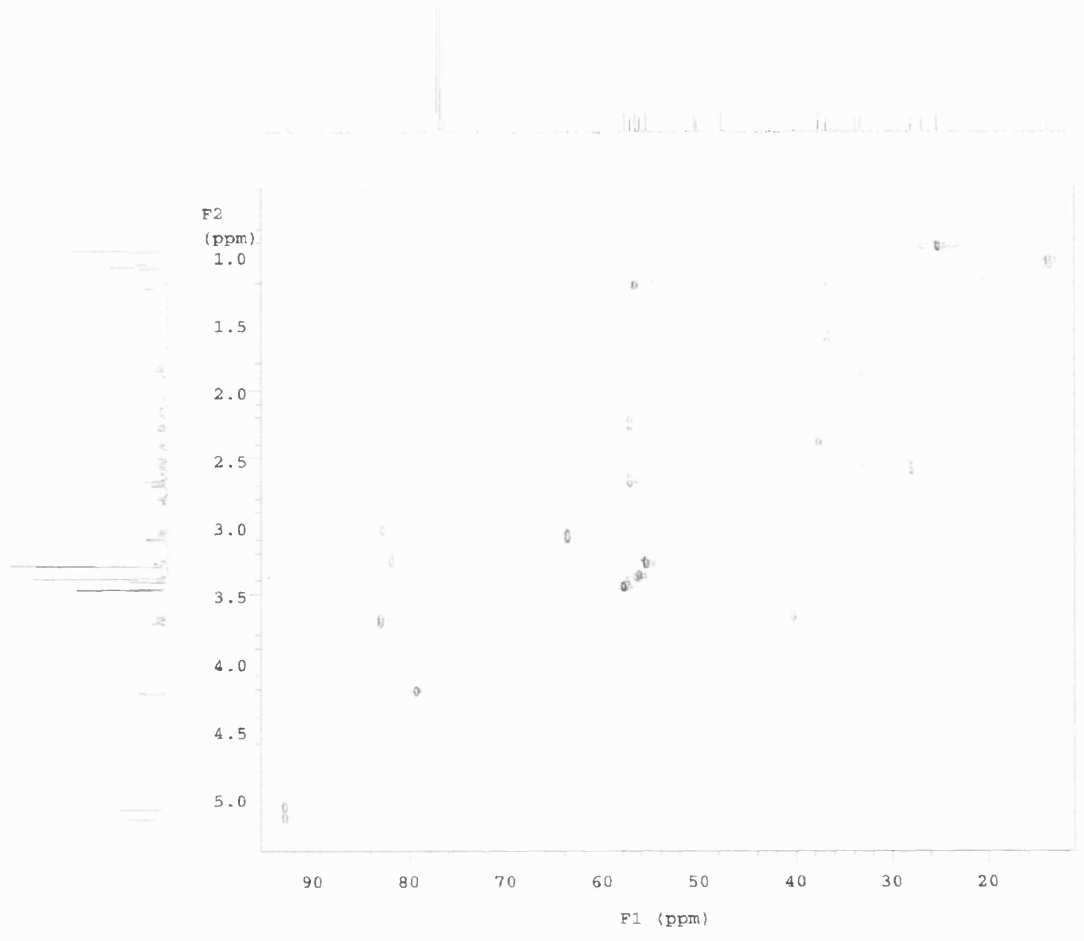


Figure 3.4 HMQC spectrum of delpheline

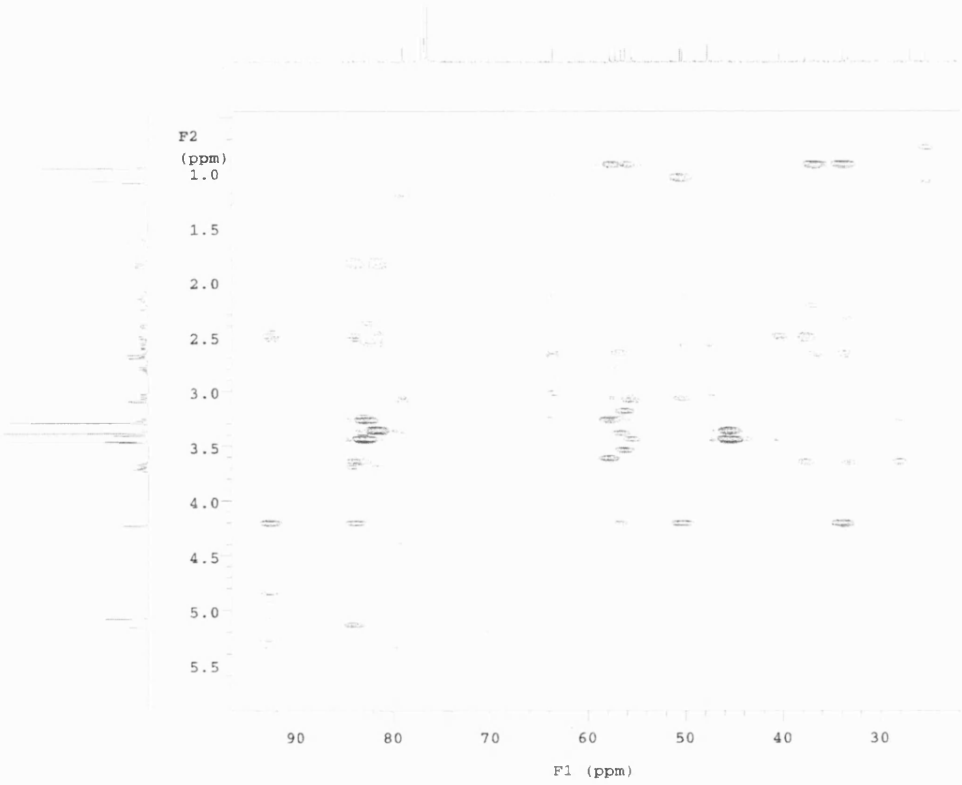


Figure 3.5 HMBC spectrum of delpheline

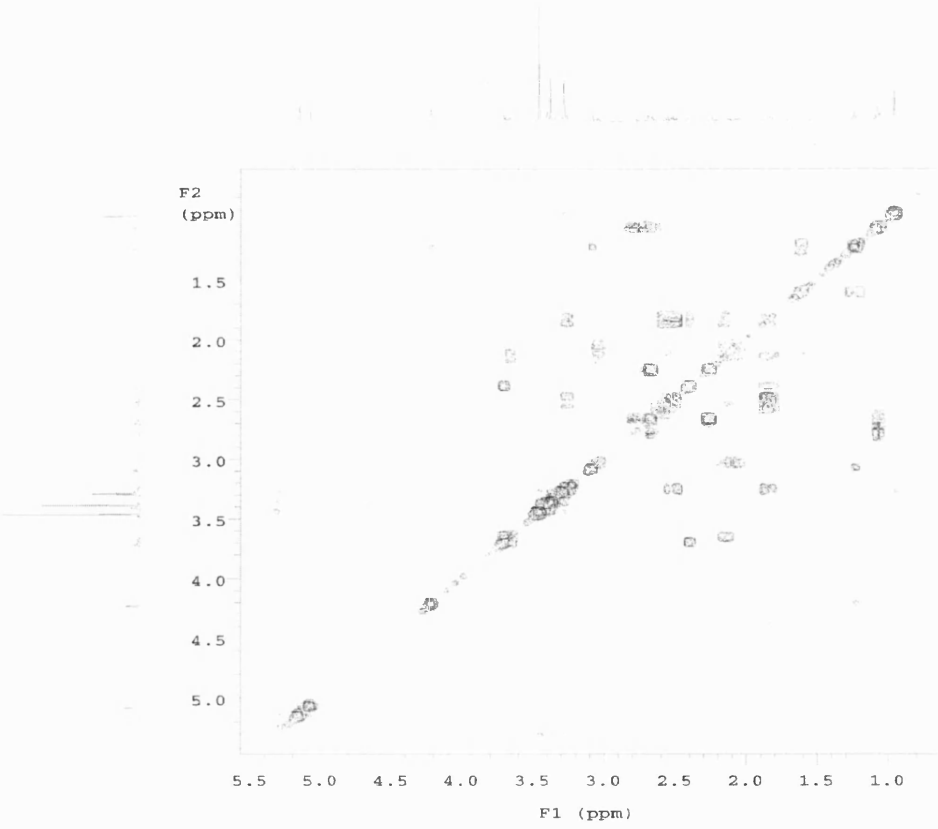


Figure 3.6 COSY spectrum of delpheline

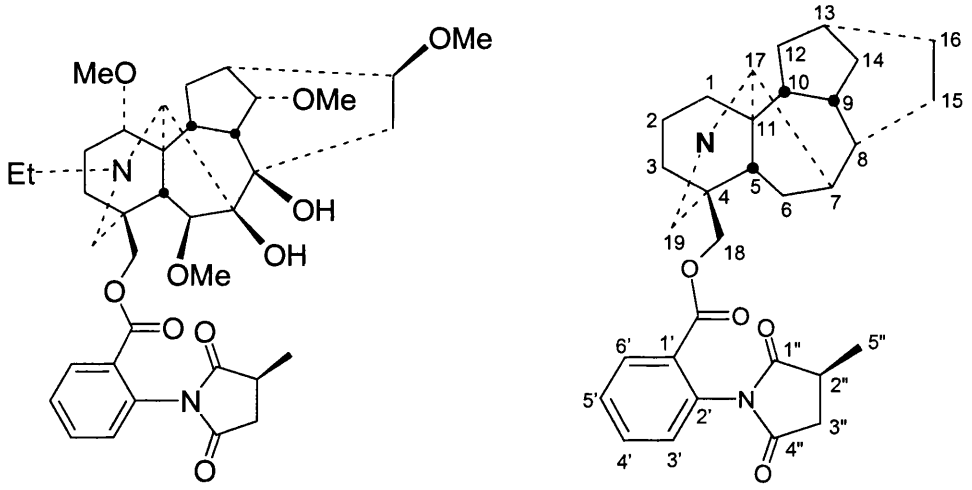
Table 1 NMR spectral data for delpheline

Carbon	δ (ppm)	Correlated protons	
		^{13}C-^{1}H (one bond)	coupling constant (Hz)
1	82.7	3.02 (dd, H-β-1)	$J_{1\beta2\alpha} = 10$ $J_{1\beta2\beta} = 7$
2	26.7	2.07-1.98 (m, H-β-2)	
		2.21-2.08 (m, H-α-2)	
3	36.9	1.26-1.16 (m, H-β-3)	
		1.59 (ddd, H-α-3)	$J_{3\alpha3\beta} = 13$ $J_{3\alpha2\alpha} = 5$ $J_{3\alpha2\beta} = 2$
4	33.8		
5	56.6	1.22 (s, H-5)	
6	79.2	4.19 (s, H-6)	
7	92.7		
8	84.1		
9	40.3	3.67-3.60 (m, H-9)	
10	47.7	2.21-2.08 (m, H-10)	
11	50.4		
12	28.1	1.86-1.78 (m, H-β-12)	
		2.55 (dd, H-α-12)	$J_{12\alpha12\beta} = 15$ $J_{12\alpha10} = 5$
13	37.7	2.37 (dd, H-13)	$J_{13,14} = 7$ $J_{13,12\beta} = 5$
14	83.0	3.71-3.64 (m, H-14)	
15	33.4	1.86-1.78 (m, H-β-15)	
		2.49 (dd, H-α-15)	$J_{15\alpha15\beta} = 15$ $J_{15\alpha16} = 9$
16	81.8	3.25-3.19 (m, H-16)	
17	63.6	3.08 (br s, H-17)	
18	25.3	0.93 (s, H_3-18)	
19	57.4	2.24 (d, H-β-19)	$J_{19\beta19\alpha} = 12$
		2.69-2.60 (m, H-α-19)	
NCH_2CH_3	50.6	2.69-2.60 (m, 1H of NCH_2CH_3)	
		2.77 (dd, 1H of NCH_2CH_3)	$J_{NCH2CH3} = 7$ $J_{NCH2,NCH2} = 12$
NCH_2CH_3	13.8	1.06 (t, NCH_2CH_3)	$J_{NCH2CH3} = 7$
$C(1)OCH_3$	55.5	3.26 (s, $C(1)OCH_3$)	
		3.34 (s, C(6)OH)	
$C(14)OCH_3$	57.8	3.43 (s, $C(14)OCH_3$)	

Carbon	δ (ppm)	Correlated protons	
		^{13}C-^{1}H (one bond)	coupling constant (Hz)
C(16)OCH_3	56.3	3.35 (s, C(16)OCH_3)	
OCH_2O	92.9	5.05 (AB d, OCH_αO)	
		5.13 (AB d, OCH_βO)	

Only two complete NMR assignments for norditerpenoid alkaloid containing 7,8-methylene-dioxy substitution have been published, both for delpheline.[138, 140] However, these two assignments disagree. Pelletier and co-workers[140] reassigned a number of ^{1}H NMR shifts of Bando and co-workers,[138] and our results are in agreement with the revisions of Joshi and co-workers.[140] The ^{1}H and ^{13}C NMR spectra of delpheline can be confidently employed, as a template, in the assignment of other 7,8-methylenedioxy substituted norditerpenoid alkaloids.

3.3.2. Methyllycaconitine (MLA)



4

MLA was first isolated from the seeds of *D. elatum*.[17] The spot with $R_f = 0.31$ by TLC (cyclohexane:chloroform:diethylamine 5:4:1) was MLA, identical with an authentic sample of MLA. A comprehensive set of spectroscopic data was again obtained for and the non-crystallisable foam was confirmed to be the alkaloid MLA. The ^{1}H NMR spectrum (400 MHz) and ^{13}C NMR spectrum (100 MHz) are shown in Figures 3.7 and 3.8 and Figures 3.9 – 3.12 show DEPT, COSY, HMQC and HMBC spectra. In Table 2 all the NMR spectral analysis for MLA can be found. Our spectra show that two protons at C-18 display some AB character.

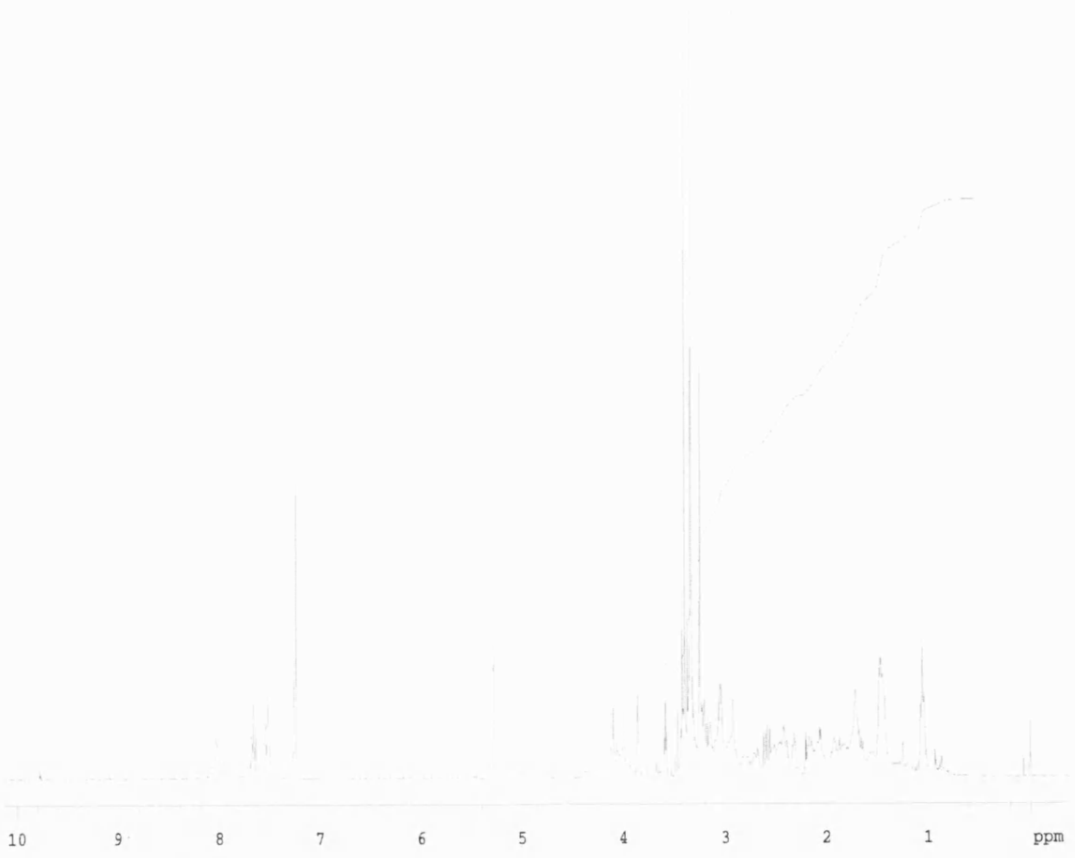


Figure 3.7 ^{1}H-NMR spectrum of MLA

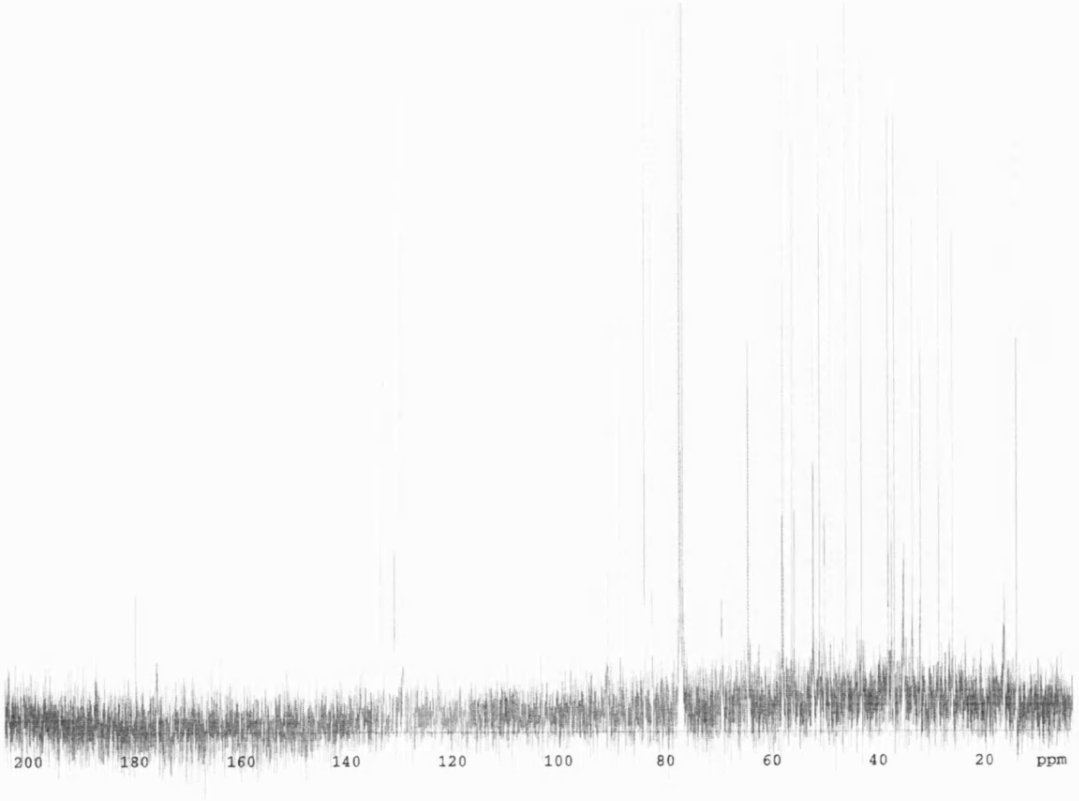


Figure 3.8 ^{13}C-NMR spectrum of MLA

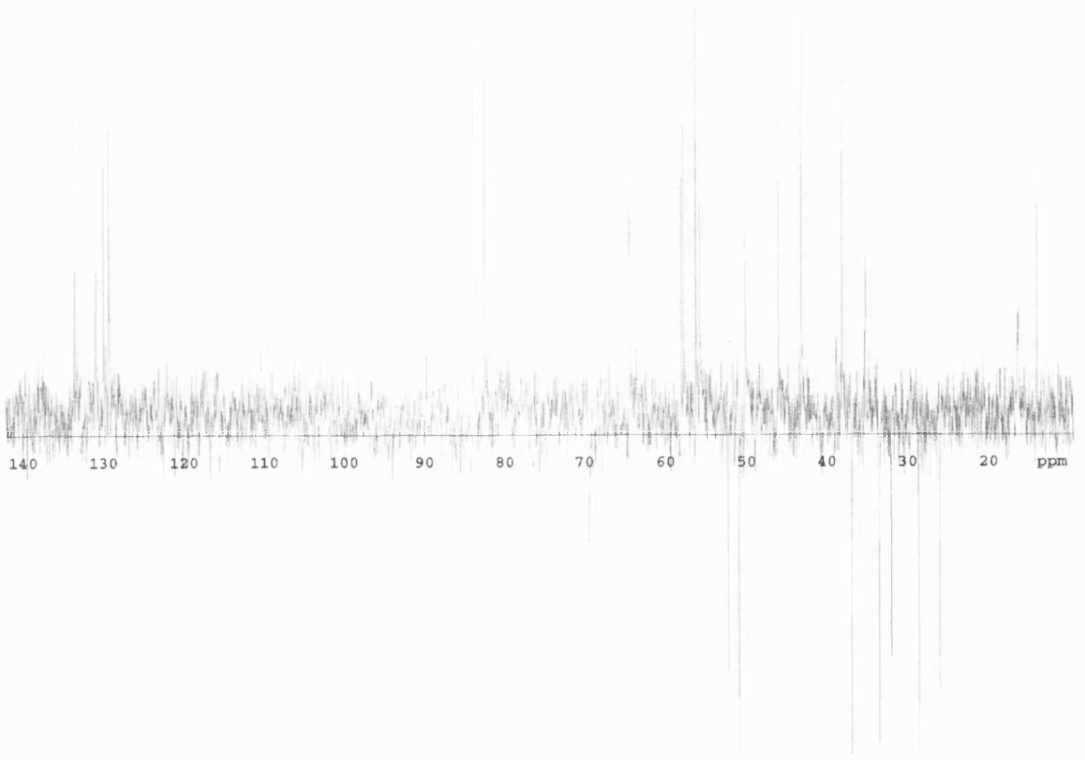


Figure 3.9 DEPT spectrum of MLA

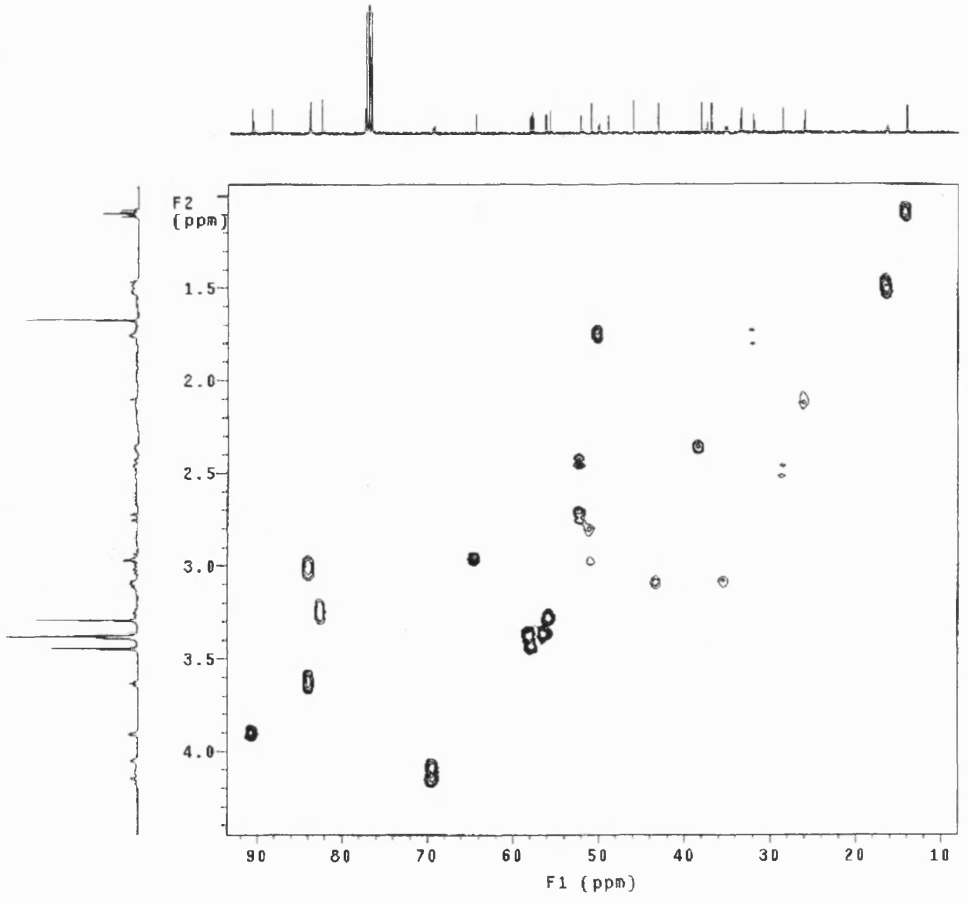


Figure 3.10 HMQC spectrum of MLA

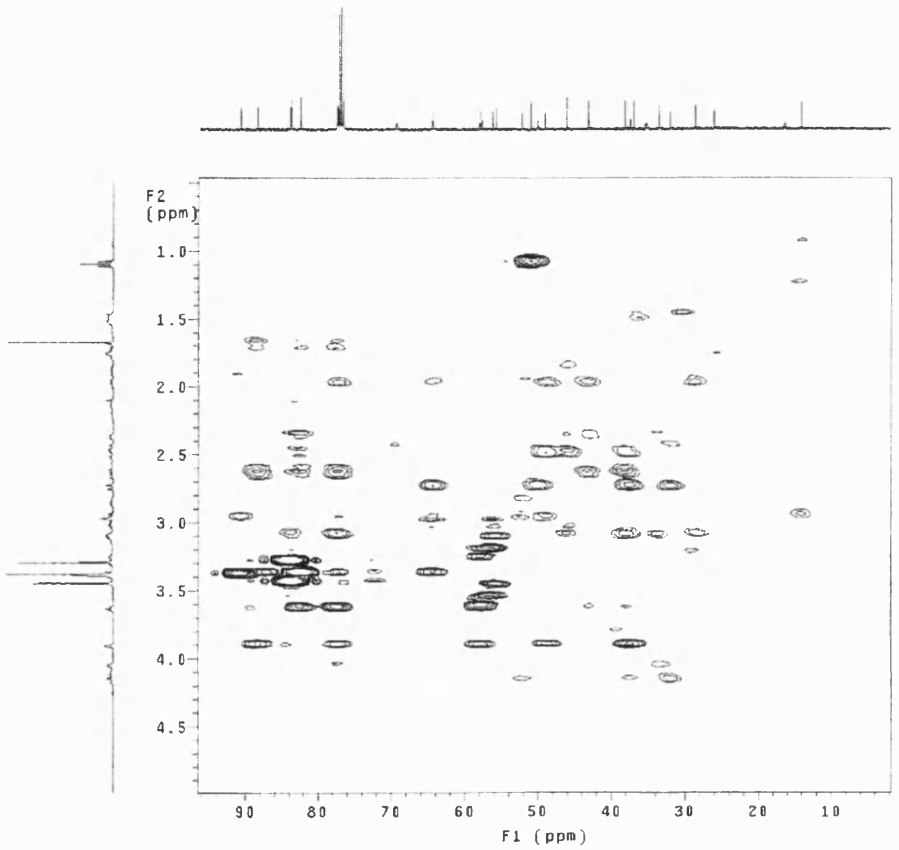


Figure 3.11 HMBC spectrum of MLA

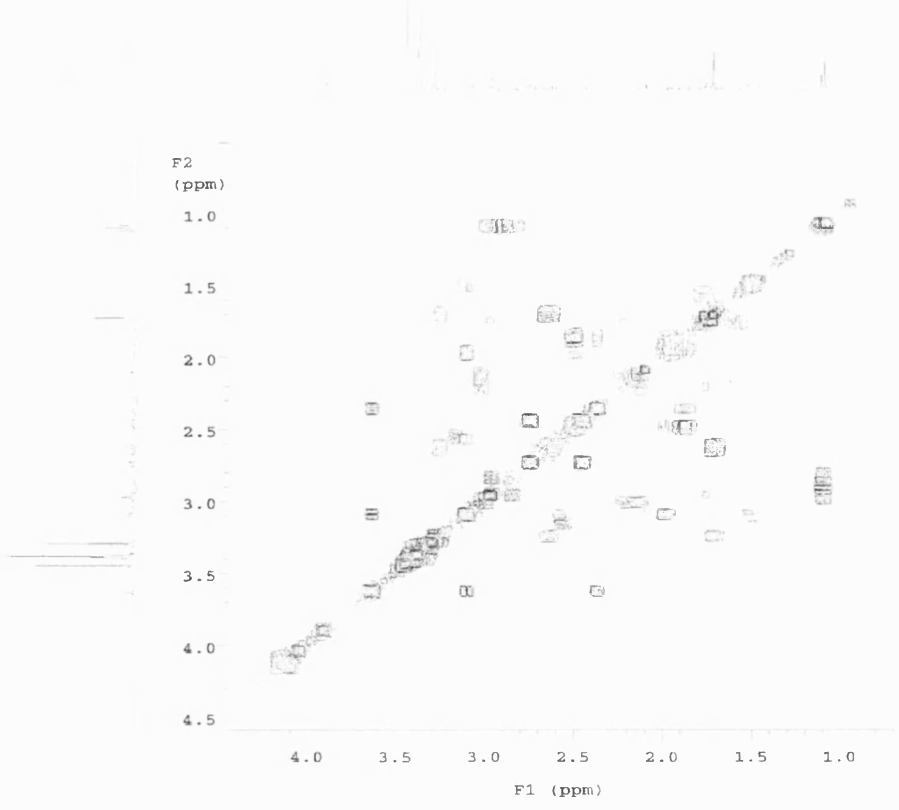


Figure 3.12 COSY spectrum of MLA

As seen for delpheline, the diagnostic region for the methoxy signals overlapped the C-19 signal which is split in the HMQC spectrum (Figure 3.11) to reflect correlation with the two different proton environments for H-α-19 and H-β-19. Also overlapping, in the region of the ^{13}C NMR spectrum (Figure 3.8), is the C-5 signal. The protons of C(1)-OCH_3 are located by the coupling of C(1)-α-OCH_3 to H-α-3 in the COSY spectrum (Figure 3.10). The HMQC spectrum (Figure 3.11) was important in assigning the methoxy groups, showing crosspeaks connecting C-16 to C(16)-OCH_3, C-6 to C(6)-OCH_3, C(16)-OCH_3 to H-6 and C-11 to C(1)-α-OCH_3. By elimination, therefore, C(14)OCH_3 can be assigned, even by deuterium exchange, possible because of heavy overlapping peaks.

Six of the signal in the ^{13}C (Figure 3.8) and DEPT ^{13}C (Figure 3.9) were in the aromatic region and could be correlated to the corresponding four protons of the anthranoyl ester group in the HMQC spectrum (Figure 3.11). Another assignment of particular significance in the ^{13}C NMR spectrum (Figure 3.8) is 164.1 for carbonyl carbon.[141] The ^{1}H NMR spectrum (Figure 3.7) showed a pattern, consisting of two triplets (dt) and two doublets (dd) in the aromatic region, as might have been expected. The meta coupling constants from the expansion of the ^{1}H NMR spectrum (400 MHz) were successfully shown. In the COSY spectrum (Figure 3.10) 3 strong ortho cross-spots and 2 weaker ones for the meta interactions were seen. The most downfield proton would be expected to be the one bonded to the carbon adjacent to the carbon attached to the carbonyl, that is H-6'. Hence it was possible to assign all protons and associated coupling constants and ,therefore, carbons by using the HMQC spectrum (Figure 3.11). The proton assignments agree with the trend for other 2-aminobenzoate esters and (*N*-acetyl)-2-aminobenzoate esters.[119, 144] We are confident of all the ^{13}C NMR assignments reported here, but they are not in agreement with the literature.[145, 146]

The crucial region of δ1.5 ppm in the ^{1}H NMR spectrum (Figure 3.7) of the aromatic containing alkaloid, where the signal for the methyl group of the imide ring is expected, showed an extremely broad signal (doublet, $J = 8$ Hz) at δ 1.47 ppm so further supporting the evidence for the presence of MLA ($J = 6$ Hz).[147] In the literature, there is some controversy as to the assignment of the carbons of the methylsuccinimido group.[3, 145-148] This seems extraordinary due to the clear indication given by the DEPT as to whether a carbon is a quaternary, methine, methylene, or methyl carbon. In the ^{13}C NMR spectrum (Figure 3.8) observe 179.8 (C-1"), 175.8 (C-4"), 37.0 (C-3"), 35.2 (C-2"), and 16.4 ppm (C-5"). Geminal cross-spots are observed in the COSY spectrum (Figure 3.10) and both two protons of H-3" to H-2" and in addition, some coupling to H-5" is seen.

For ring A, an A_2B_2X spin system is observed where X is H-β-1, A_2 is H_2-3, and B_2 is H_2-2. the COSY spectrum (Figure 3.10) was helpful, showing crosspeaks between H-β-1 and H-α-2, H-β-1 and H-β-2, H-β-1 and H-α-3. Other coupling seen in the COSY spectrum (Figure 3.10) connect H-α-2 to H-β-2, H-α-2 to H-α-3, H-β-2 to H-β-3, H-β-2 to H-α-3, and possibly H-α-3 to H-β-3. Other couplings seen were NCH_2CH_3 or H-α-19 to H-α-2. C-3 was assigned by elimination as 32.1 ppm over C-15, using HMQC and HMBC spectrum (Figures 3.11 and 3.12), as H-β-15 couples to both C-9 and C-15. For ring B, an AX pattern is observed. For ring C, an ABX system of H-13 (B), H-9 (A), and H-14 (X) are observed. The couplings observed include H-9 to H-14 and H-13 to H-14, but not H-9 to H-13. On inspection of the fine structure of the expansion of the ^1H NMR spectrum (Figure 3.7), we see $J_{13,14}$ = 5 Hz and $J_{9,14}$ = 5 Hz), indicating that the signal for H-14 is a clear doublet of doublets. Crosspeaks in the COSY spectrum (Figure 3.10) provided evidence for a *cis* ring junction at C-9 to C-10. An ABC system is caused by H_2-12 (A and C) and H-10 (B).

Table 2 NMR spectral data of MLA

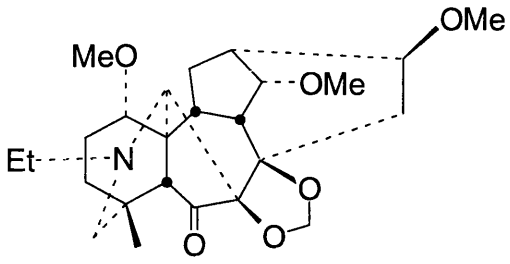
Carbon	δ (ppm)	Correlated protons	
		^{13}C-^1H (one bond)	coupling constant (Hz)
1	83.9	2.95-2.92 (m, H-β-1)	
2	26.1	2.15-2.03 (m, H-β-2)	
		2.20-2.15 (m, H-α-2)	
3	32.1	1.58-1.52 (m, H-β-3)	
		1.78-1.72 (m, H-α-3)	
4	37.6		
5	50.3	1.70-1.64 (m, H-5)	
6	90.8	3.85 (s, H-6)	
7	88.5		
8	77.5		
9	43.2	3.10-3.03 (m, H-9)	
10	46.1	1.98-1.90 (m, H-10)	
11	49.1		
12	28.7	1.98-1.90 (m, H-β-12)	
		2.50-2.45 (m, H-α-12)	
13	38.0	2.35-2.31 (m, H-13)	
14	83.0	3.60 (d, H-14)	$J_{14,13}$ = 5 $J_{14,9}$ = 5
15	33.6	1.70-1.64 (m, H-β-15)	
		2.64-2.56 (m, H-α-15)	
16	82.6	3.24-3.17 (m, H-16)	
17	64.5	2.95-2.92 (br s, H-17)	

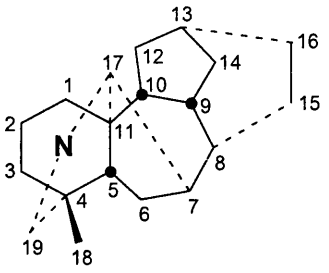
Carbon	δ (ppm)	Correlated protons	
		^{13}C-^{1}H (one bond)	coupling constant (Hz)
18	25.3	4.15-4.00 (m, H-β-18)	
		4.15-4.00 (m, H-α-18)	
19	52.4	2.45-2.38 (m, H-β-19)	
		2.69-2.60 (m, H-α-19)	
NCH_2CH_3	50.9	2.73-2.69 (m, 1H of NCH_2CH_3)	
		2.95-2.92 (m, 1H of NCH_2CH_3)	
NCH_2CH_3	14.1	1.07 (t, NCH_2CH_3)	$J_{NCH2CH3} = 7$
$C(1)OCH_3$	55.8	3.28 (s, $C(1)OCH_3$)	
$C(6)OCH_3$	58.1	3.45 (s, $C(6)OCH_3$)	
		(s, C(7)OH)	
		(s, C(8)OH)	
$C(14)OCH_3$	57.8	3.42 (s, $C(14)OCH_3$)	
$C(16)OCH_3$	56.3	3.38 (s, $C(16)OCH_3$)	
C=O	164.1		
1'	126.9		
2'	133.1		
3'	130.0	7.30 (d, H-3')	$J_{3'4'} = 8$
4'	133.7	7.70 (t, H-4')	$J_{4'3'} = 8$ $J_{4'5'} = 8$
5'	129.4	7.55 (t, H-5')	$J_{5'4'} = 8$ $J_{5'6'} = 8$
6'	131.0	8.05 (d, H-6')	$J_{6'5'} = 8$
1"	179.8		
2"	35.2	3.02-2.96 (m, H-2")	
3"	37.0	2.56-2.50 (m, H-3")	
		3.10-3.03 (m, H-3")	
4"	175.8		
5"	16.4	1.47 (br d, H_3-5")	$J_{5"2"} = 8$

The HMBC spectrum (Figure 3.12) is very informative in allowing the assignment of C-10 and C-13 based on the coupling of C-10 to H-13 and C-16 to H-13). Crosspeaks in the COSY spectrum (Figure 3.10) confirmed the assignment and stereochemistry of H-β-12 and H-β-13. For ring D, an ABX system is applied for H_2-15 (A and B) and H-16 (X). The observed couplings include H-α-15 to H-β-15, H-α-15 to H-16, and H-β-15 to H-16). Other couplings seen were H-α-12 to H-16. For ring E, C-17 and C-19 are bridged by a nitrogen atom resulting in relatively downfield resonances of H-17 and H_2-19. H_2-19 are observed at δ2.45-2.38 ppm and δ2.73-2.69 ppm showing some AB character. The methylene protons of

N-ethyl side-chain appear as a multiplet at δ 2.95-2.69 ppm rather than displaying a clear AB system. The terminal methyl group of *N*-ethyl side-chain appears as a triplet at δ 1.07 ppm ($NC\mathit{H}_2CH_3$ coupling to $NCH_2C\mathit{H}_3$ as expected).

3.3.3. Pacinine 118

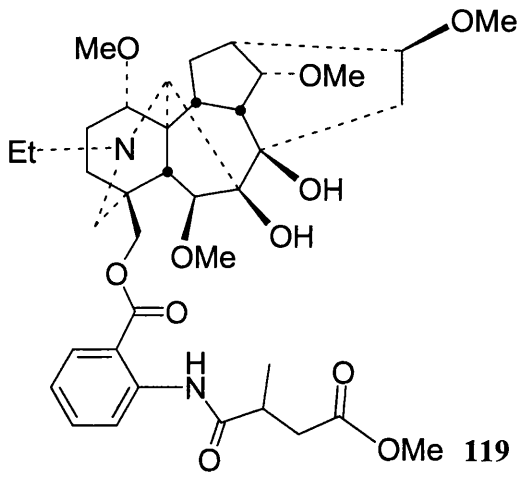




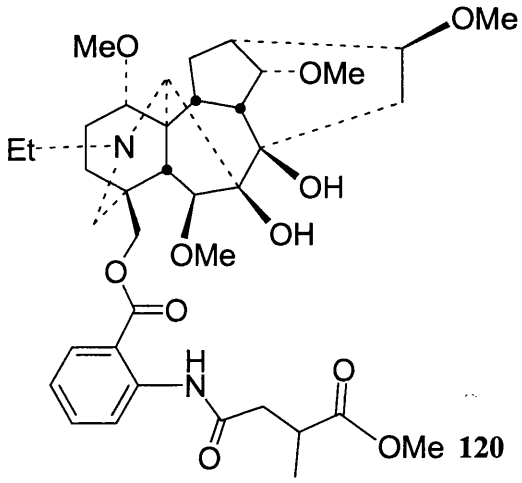
118

Pacinine **118** was purified to homogeneity from crude ethanolic extracts of the seeds of Delphinium cv Pacific Giant by flash column chromatography on acidic alumina, the fraction from 2% methanol in diethyl ether yielded pacinine **118** (and fraction 8, from 5% methanol in diethyl ether also yielded MLA **4**). The methylenedioxy signals resonated at: 5.51 (1H, AB d, 1H of $OC\mathit{H}_2O$) and 5.07 (1H, AB d, 1H of $OC\mathit{H}_2O$) ppm. The C6 proton (and therefore its signal) had disappeared in this ketone of delpheline **1**.

3.3.4. Delavaines A and B 119 and 120



119

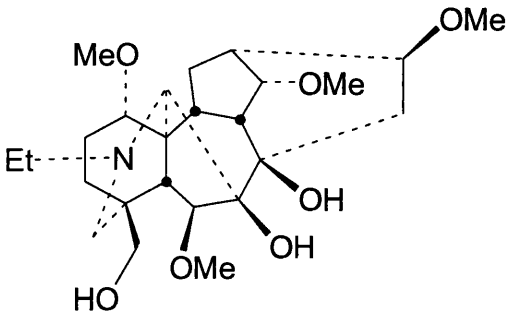


120

These compounds **119** and **120** eluted after MLA from the crude ethanolic extract and they were purified further by flash column chromatography on neutral alumina, until they were homogenous by TLC $R_f = 0.15$ (where MLA typically shows $R_f = 0.31$) (see Experimental for details), but still a 3:2 mixture by ^1H NMR integration. When the chromatography was performed with 2-propanol (on the ethanolic extract), the same compounds were isolated, with identical HRMS (715.3782 Da). We therefore conclude that these methyl esters are indeed natural products and not artefacts from methanol used during the isolation.

MLA **4** is a selective competitive antagonist at α7 sub-type nAChR. From collaborative studies, the biological activities of delpheline **1**, pacinine **118** (the B-ring C6-ketone of delpheline), and the delavaines A and B **119** and **120** (as a 3:2 mixture), were determined in competitive α-bungarotoxin binding assays for α7 nAChR in rat brain membranes. The succinimide ring opened delavaines A and B **119** and **120** were potent ligands (IC_{50} = 50 nM, cf MLA IC_{50} = ~1-2 nM), whereas delpheline **1** and pacinine **118**, both lacking the C18-anthranilate ester moiety, displayed only modest activity at α7 nAChR (IC_{50} = ~1 μM).

3.3.5. Lycoctonine 6



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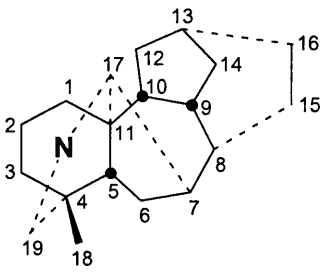


Lycoctonine **6** was obtained in crystalline form from the saponification of MLA. Its assignment followed for a norditerpenoid skeleton and the substituents whereby the ^{1}H NMR spectrum (400 MHz) and ^{13}C NMR spectrum (100 MHz), DEPT, COSY, HMQC, and HMBC spectra shown in Figures 3.13-3.18. Comparison of our data was made with literature values.[3, 145, 147, 148] Table 3 shows the information obtained from the spectra.

From the COSY spectrum (Figure 3.16) coupling for each of the relevant methine protons to the associated methoxyl protons were seen along with cross-spots connecting C(1)-α-O*CH*$_3$ to H-α-3 and C(16)-β-O*CH*$_3$ to H-β-14, thus allowing confident assignment of the methoxy proton. The C(8)-β-OH signal at δ 4.08 ppm was located by its interaction with H-β-15 and H-β-9 and C(7)-β-OH at δ1.75 ppm was found to interact with both H-6 and H-17 and C(8)-OH at δ1.59-1.47 ppm was interacted with H-β-9 and H-β-18.

Before D_2O exchange, the signals from the C*H*$_2$OH hydrogen atoms (H_2-18) are seen as a multiplet due to coupling between protons (H_2-18) and a hydroxyl proton. This showed AB character with a shift difference of A (at δ 3.68-3.59 ppm) and B (at δ 3.35 ppm) of δ0.28 ppm. H-β-18 and H-α-18 can be seen to interact with a coupling constant of J = 9 Hz. After D_2O exchange, the signals appear as an AB quartet at δ 4.00 ppm and δ 4.24 ppm with J = 12 Hz, because this is a prochiral centre. The assignment of the hydroxyl protons was possible by careful examination of the integration before and after D_2O exchange.

Table 3 NMR spectral data of lycoctonine

Carbon	δ (ppm)	Correlated protons	
		^{13}C-^{1}H (one bond)	coupling constant (Hz)
1	84.2	2.96-2.88 (m, H-β-1)	
2	26.1	2.20-2.01 (m, H-β-2)	
		2.20-2.01 (m, H-α-2)	
3	31.6	1.59-1.47 (m, H-β-3)	
		1.69-1.63 (m, H-α-3)	
4	38.5		
5	46.1	1.94-1.78 (m, H-5)	
6	90.6	3.84 (s, H-6)	
7	88.4		
8	77.4		
9	49.5	1.69-1.63 (m, H-9)	
10	38.0	2.33 (dd, H-10)	$J_{10,12\alpha} = 5$ $J_{10,14} = 5$ $J_{10,12\beta} = 7$
11	48.8		
12	28.7	1.94-1.78 (m, H-β-12)	
		2.43 (dd, H-α-12)	$J_{12\alpha12\beta} = 14$ $J_{12\alpha10} = 5$ $J_{12\alpha13} = 5$
13	43.2	3.08-3.05 (m, H-13)	
14	83.9	3.68-3.59 (m, H-14)	
15	33.5	1.69-1.63 (m, H-β-15)	
		2.63-2.57 (m, H-α-15)	
16	82.5	3.21 (d, H-16)	$J_{16,15\alpha} = 9$ $J_{16,15\beta} = 8$
17	64.8	2.96-2.88 (m, H-17)	
18	67.7	3.35 (d, H-β-18)	$J_{18\beta18\alpha} = 9$
		3.68-3.59 (m, H-α-18)	
19	52.5	2.27 (d, H-β-19)	$J_{19\beta19\alpha} = 12$
		2.63-2.57 (m, H-α-19)	
NCH_2CH_3	51.1	2.87-2.75 (m, 1H of NCH_2CH_3)	
		2.96-2.88 (m, 1H of NCH_2CH_3)	
NCH_2CH_3	14.1	1.04 (t, NCH_2CH_3)	$J_{NCH2CH3} = 7$
C(1)OCH_3	55.8	3.25 (s, C(1)OCH_3)	
C(6)OCH_3	57.9	3.44 (s, C(6)OCH_3)	
		1.75 (br s, C(7)OH)	
		4.08 (s, C(8)OH)	
C(14)OCH_3	57.8	3.41 (s, C(14)OCH_3)	
C(16)OCH_3	56.2	3.34 (s, C(16)OCH_3)	
		1.59-1.47 (m, C(18)OH)	

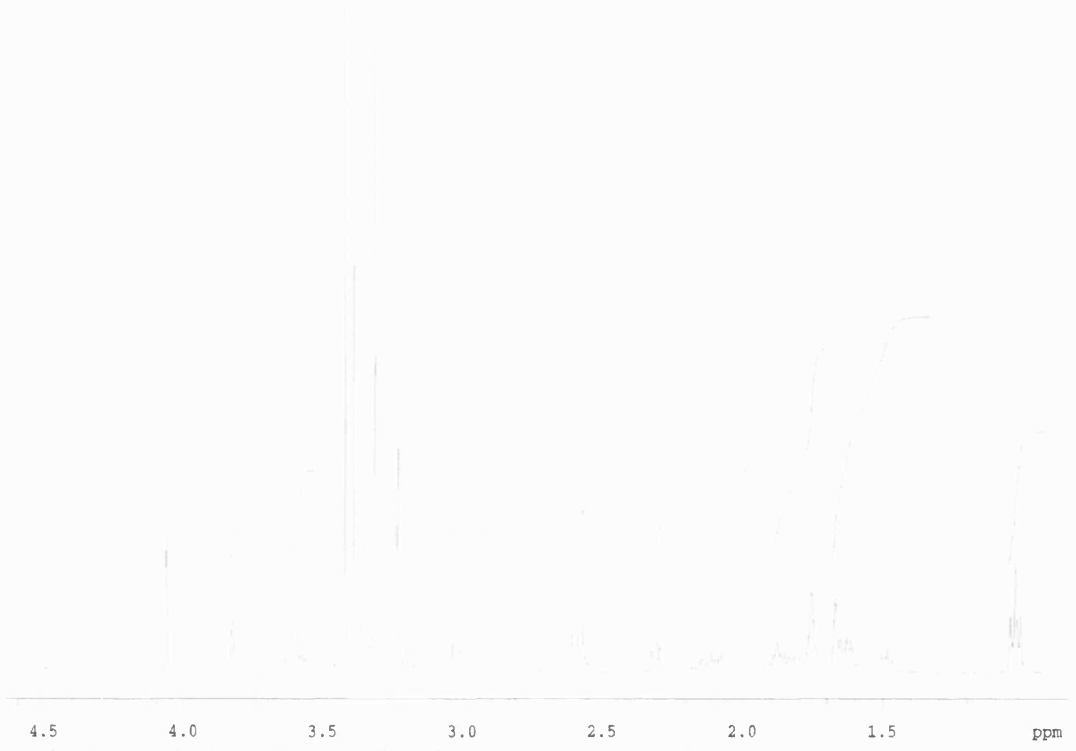


Figure 3.13 ^{1}H-NMR spectrum of lycoctonine

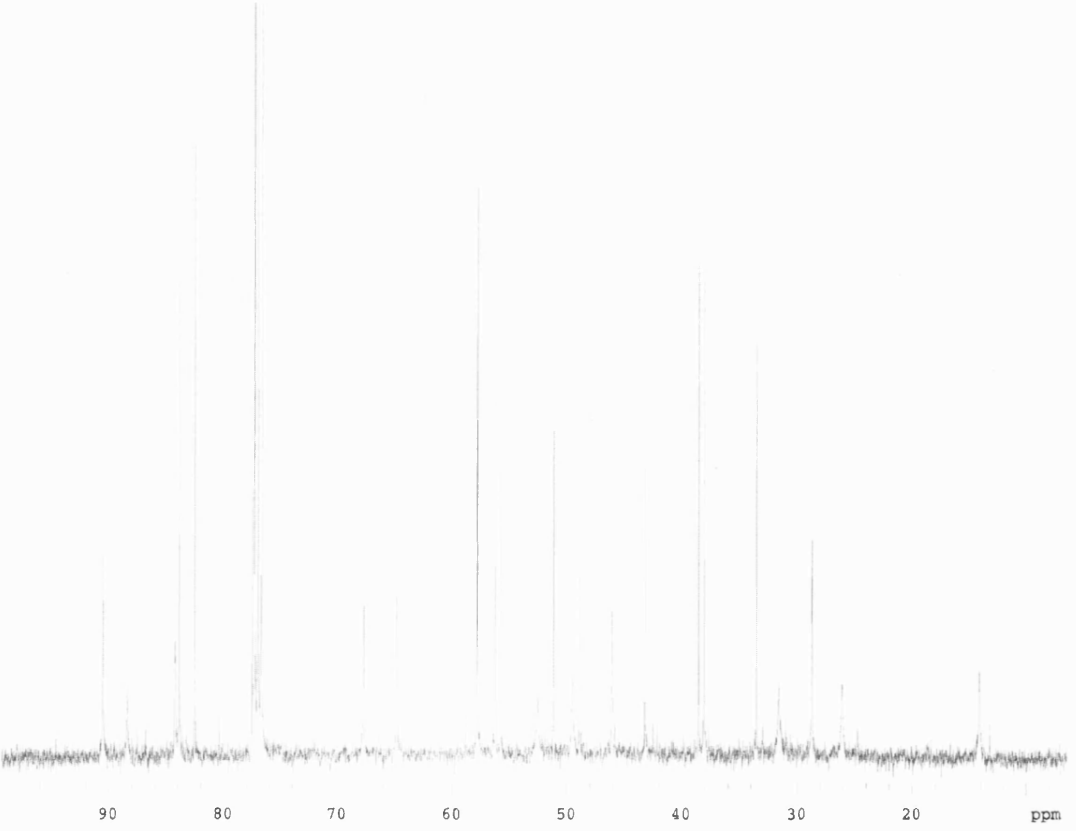


Figure 3.14 ^{13}C-NMR spectrum of lycoctonine

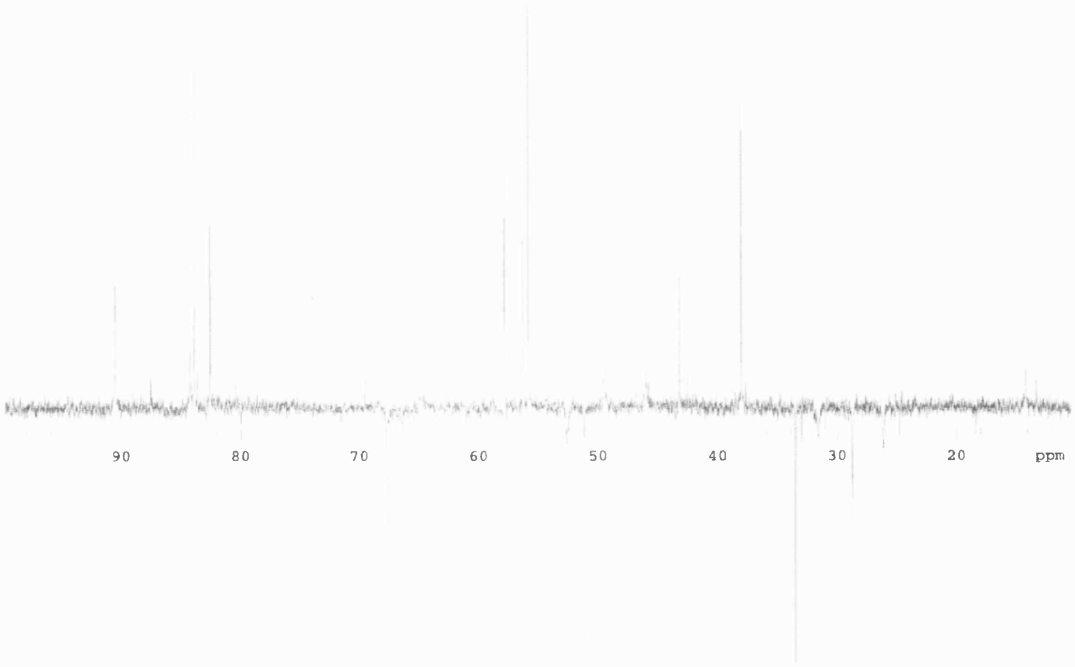


Figure 3.15 DEPT spectrum of lycoctonine

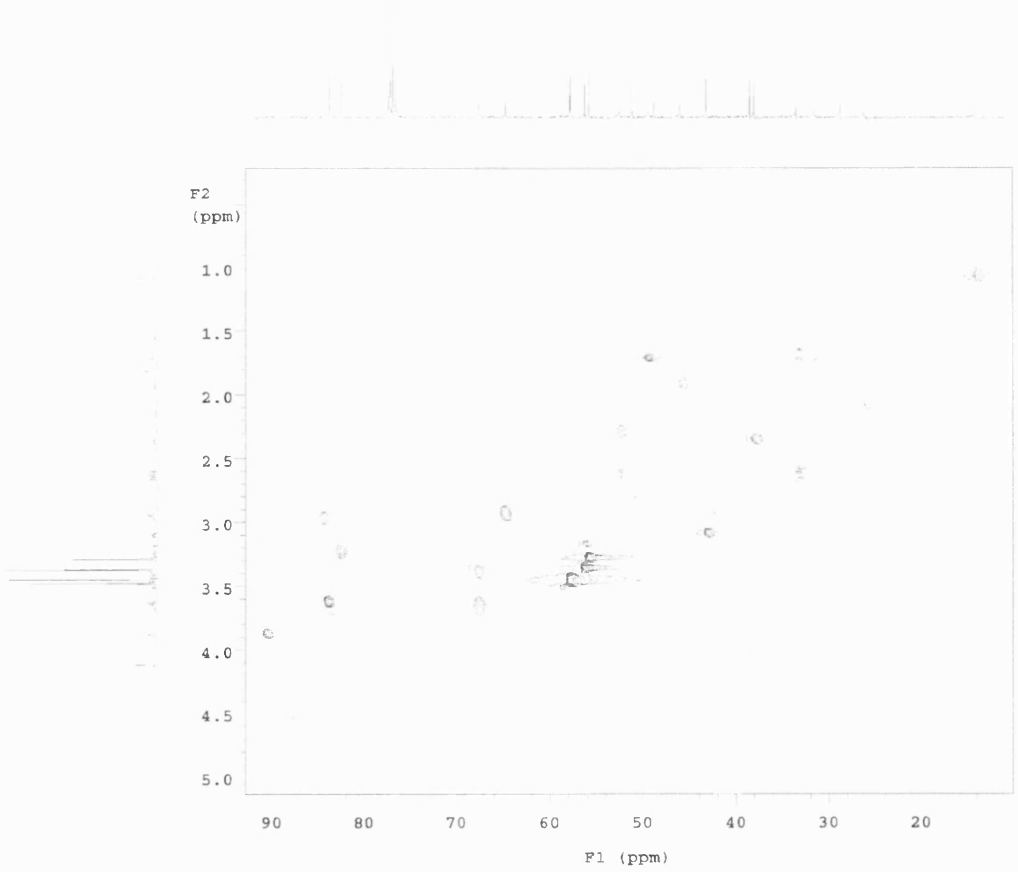


Figure 3.16 HMQC spectrum of lycoctonine

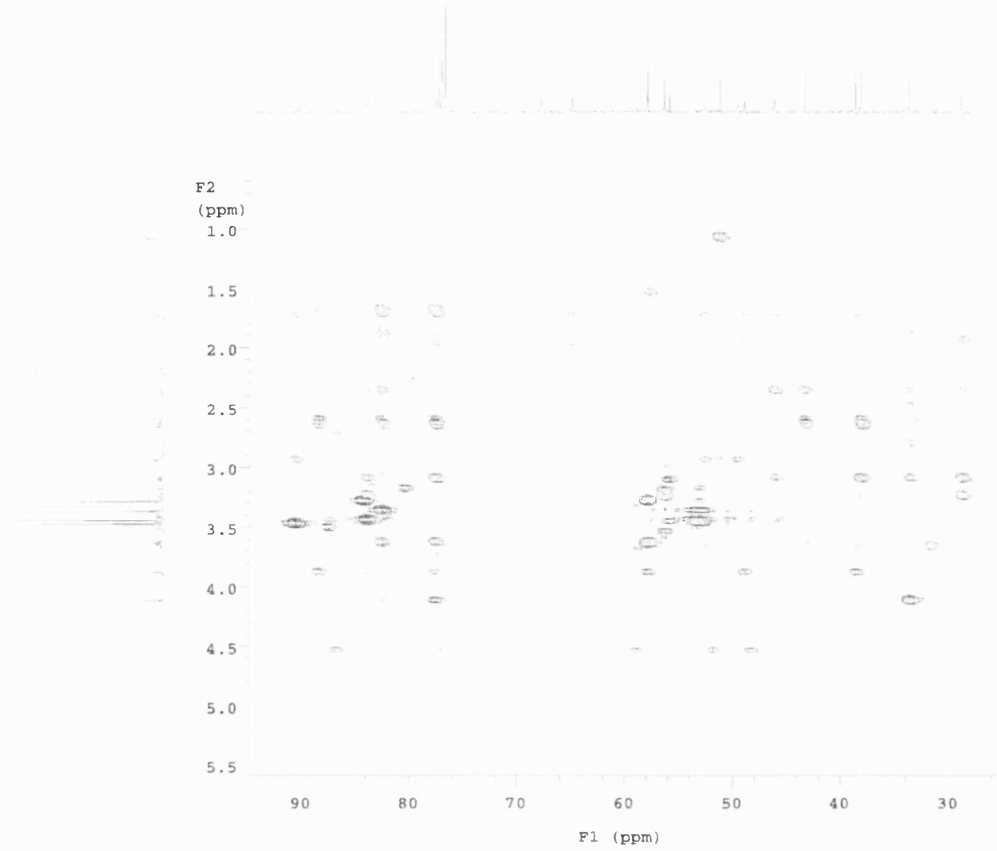


Figure 3.17 HMBC spectrum of lycoctonine

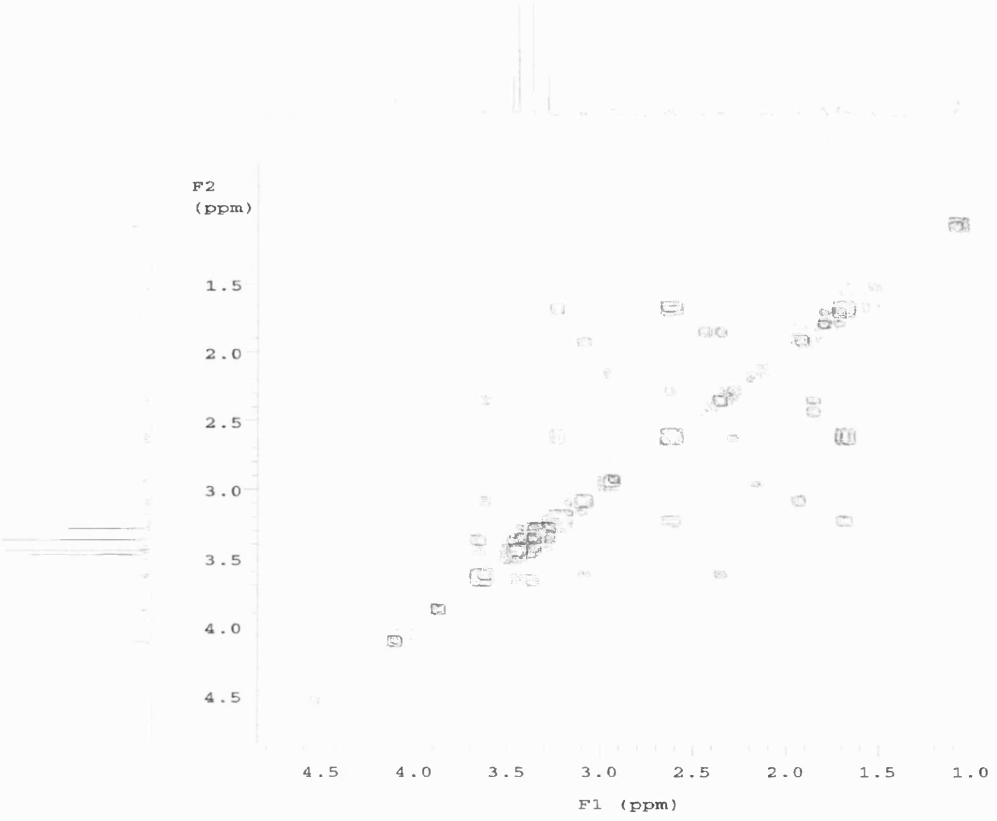
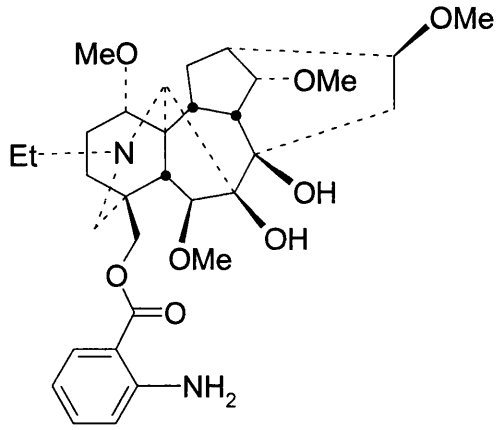


Figure 3.18 COSY spectrum of lycoctonine

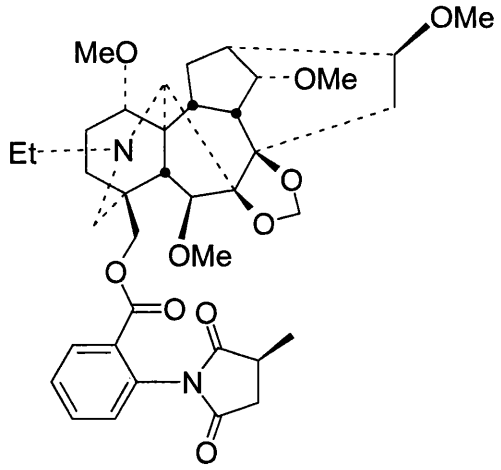
3.3.6. Inuline (anthranoyl lycoctonine) 7



7

Mild acid catalysed hydrolysis of MLA in 1,4-dioxane, even over 19 days at room temperature, failed to yield pure inuline, free of MLA. (It is important for any biological activity studies that there is no trace of MLA present, as with an IC_{50} of ~ 1 nM it is so potent that it will affect subsequent results. This was not to be a practical route to this alkaloid. Therefore, the neopentyl alcohol functional group of lycoctonine was esterified by reaction with isatoic anhydride catalysed by 4-(*N*,*N*-dimethylamino)pyridine to yield a pale yellow oil (37 mg, 12%), inuline **7**, homogenous by TLC (R_f = 0.68) and displaying the required HRMS: m/z of MH^+ $C_{32}H_{47}N_2O_8$ requires 587.3332, found 587.3299.

3.3.7. Elatine 3



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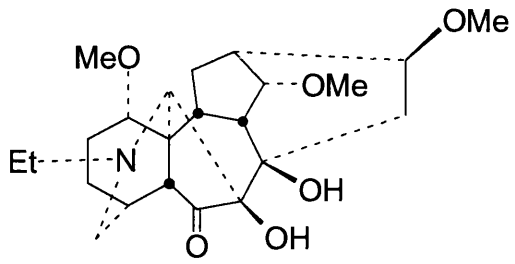
MLA was converted into elatine by formation of the methylenedioxy functional group using formaldehyde diethyl acetal and a catalytic amount of *p*-toluenesulfonic acid monohydrate under azeotropic distillation with a Dean-Stark trap for 2 h. Flash column chromatography over silica gel, using 5% methanol in dichloromethane as the eluent, finally gave elatine **3** as a yellow solid (20 mg, 7%). δ_H ($CDCl_3$) included the diagnostic 5.07 (2H, s, OCH_2O) ppm, together with HRMS: m/z of MH^+ $C_{38}H_{51}N_2O_{10}$ requires 695.3538, found 695.3532 Da.

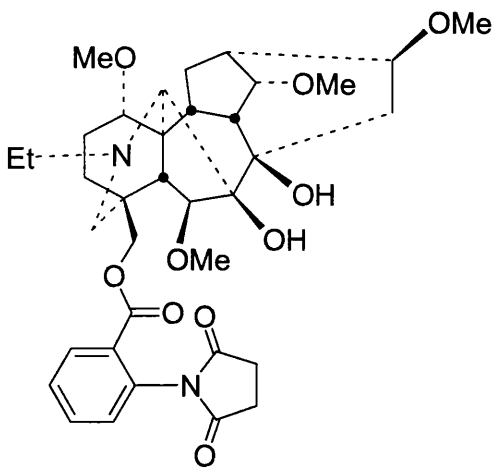
3.4. Norditerpenoid alkaloids from *Aconitum lycoctonum*

Whilst, most of studies of *A. lycoctonum* have focused on the roots, Zinurova showed that a known aconitine-type alkaloid, lappaconitine **26**, and (and C18, bis-nor) lycoctonine-type alkaloid, **140**, were isolated from the seeds of *A. septentrionale* L. (synonymous with *A. lycoctonum*).[149] In our studies, two lycoctonine type alkaloids, lycaconitine **121** and *N*-succinylanthranoyl lycoctonine **122**, were isolated from the seeds of *A. lycoctonum*.



140

Lycaconitine 121 and *N*-succinylanthranoyl lycoctonine 122



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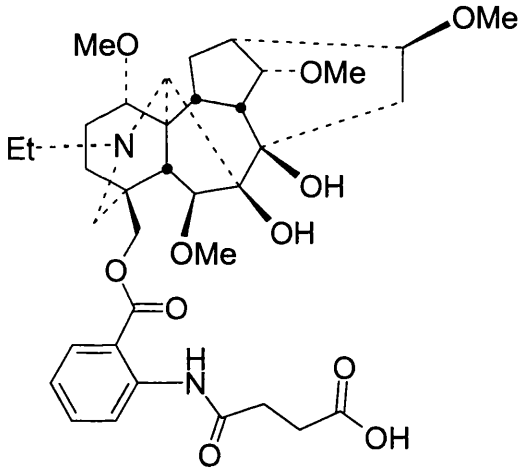

122

From ground, hexane defatted *A. lycoctonum* seeds extracted with ethanol, after acid-base cycling and purification by flash column chromatography on silica gel, a mixture (8:1 by ^{1}H NMR integration) was obtained. The major component was lycaconitine **121** δ_H 7.94 (d), 7.60 (t), 7.46 (t), and 7.17 (d) ppm assigned to its anthranilate residue, the minor component was *N*-succinylanthranoyl lycoctonine **122** δ_H 10.95 (s, carboxylic acid), 8.60 (d), 7.88 (d), 7.24 (s), and 7.01 (t) ppm assigned to its anthranilate residue. Further purification by HPLC (see Experimental for details) gave the two homogenous compounds which displayed HRMS: m/z of MH^+ found 669.3379, MH^+ of lycaconitine **121** requires 669.3387 and m/z of MH^+ found 687.3504, MH^+ of *N*-succinylanthranoyl lycoctonine **122** requires 687.3493.

3.5. X-Ray crystallography

X-Ray crystallographic studies have proved invaluable in establishing the absolute stereochemistry for a wide variety of norditerpenoid alkaloids, reviewed in 1987.[76] In 1982, the X-ray crystallographic investigations of Edwards and Przybylska focused on lycoctonine-type alkaloids, and also Codding reported the aconitine data X-ray data.[150, 151]

From a comprehensive review of X-ray analyses published since 1987, we conclude that the conformations of the rings in these alkaloids are: A and E, chair; D, half-chair or boat; C and F, envelopes; and B, boat (see Table 4).

Table 4 X-ray conformational analyses of norditerpenoid alkaloids published since 1987

Cpd	Type	Conformation						Ref
		Ring A	Ring B	Ring C	Ring D	Ring E	Ring F	
141	atypical	chair	boat	envelope	boat	distorted chair	envelope	[152]
142	atypical	boat	boat	envelope	boat	chair	distorted envelope	[153]
143	atypical	twisted boat	boat	envelope	boat	twisted boat	-	[154]
144	typical	chair	boat	envelope	boat	chair	envelope	[155]
145	atypical	boat	boat	envelope	boat	chair	envelope	[156]
146	atypical	boat	boat	envelope	boat	chair	envelope	[157]
147	atypical	boat	boat	envelope	boat	chair	envelope	[158]
148	atypical	boat	boat	envelope	boat	chair	envelope	[159]
149	typical	chair	boat	envelope	boat	chair	envelope	[77]
150	typical	chair	boat	envelope	boat	chair	envelope	[160]
151	typical	chair	boat	envelope	half-chair	chair	envelope	[161]
152	typical	chair	boat	envelope	half-chair	chair	envelope	[162]
153	typical	chair	boat	envelope	half-chair	chair	envelope	[163]
154	typical	chair	boat	envelope	half-chair	chair	envelope	[163]
155	typical	chair	boat	envelope	half-chair	chair	envelope	[164]
156	typical	chair	boat	envelope	half-chair	chair	envelope	[165]
157	typical	chair	boat	envelope	half-chair	chair	envelope	[166]
158	atypical	half-chair	boat	envelope	half-chair	chair	envelope	[167]
159	atypical	twisted	boat	envelope	boat	twisted	distorted chair	[168]

160	atypical	chair	boat	envelope	boat	half-chair	-	[169]
161	atypical	chair	boat	distorted envelope	boat	chair	distorted envelope	[170]
26	atypical	boat	boat	envelope	boat	chair	envelope	[171]
162	typical	chair	boat	envelope	boat	chair	envelope	[78]
163	atypical	boat	boat	envelope	boat	chair	distorted envelope	[172]
84	typical	chair	boat	envelope	half-chair	chair	envelope	[63]
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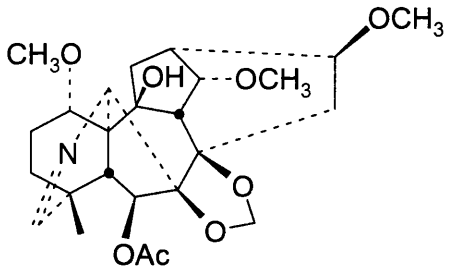

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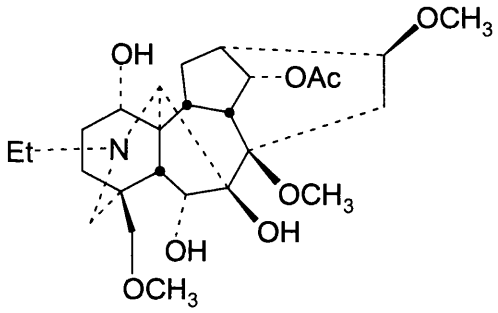

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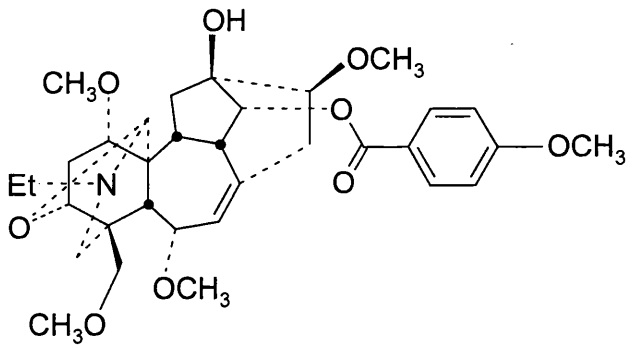

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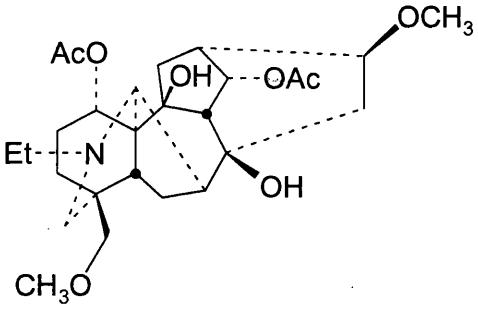

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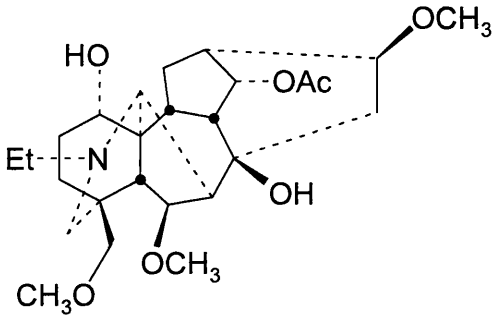

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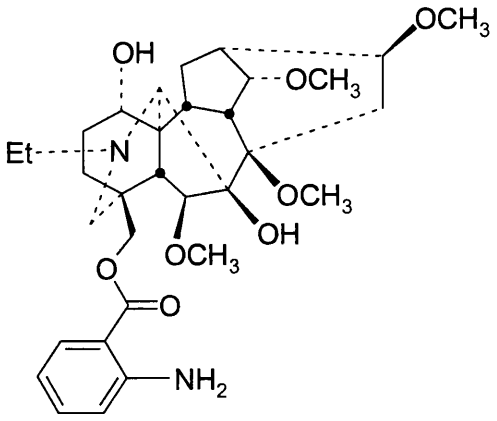

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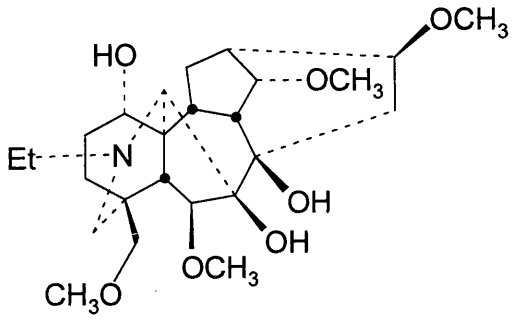

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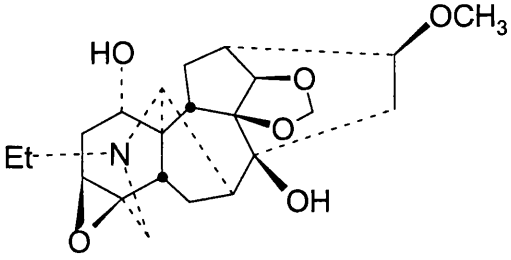

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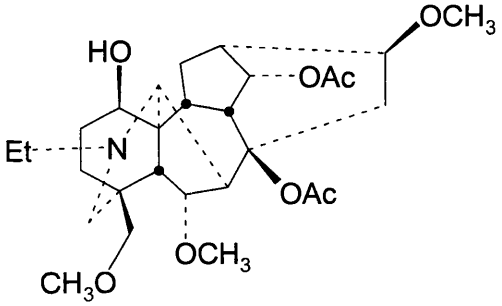

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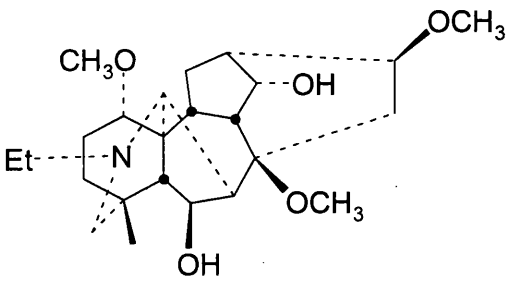

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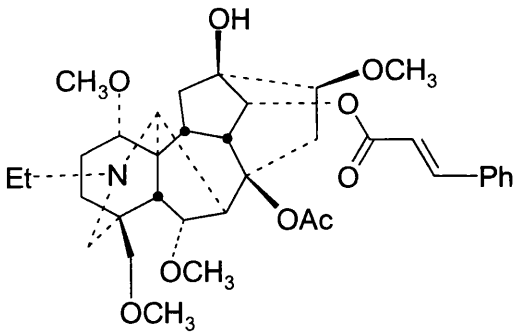

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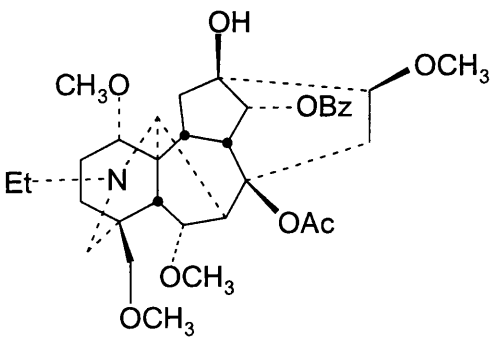

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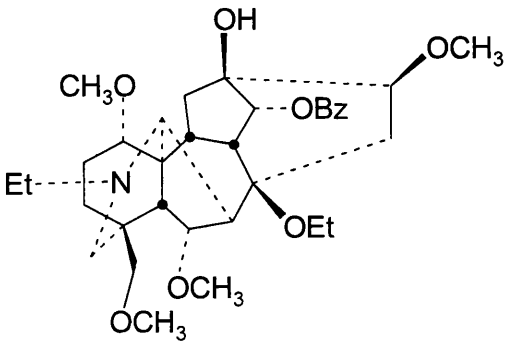

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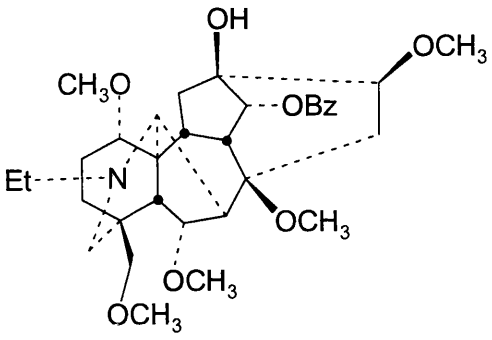

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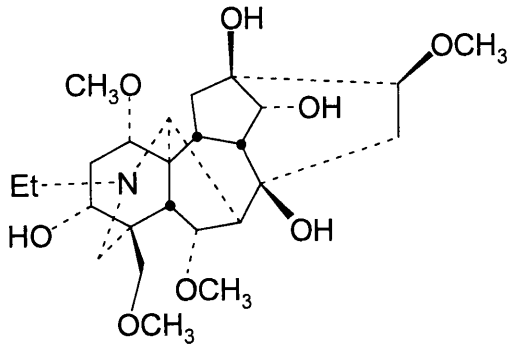

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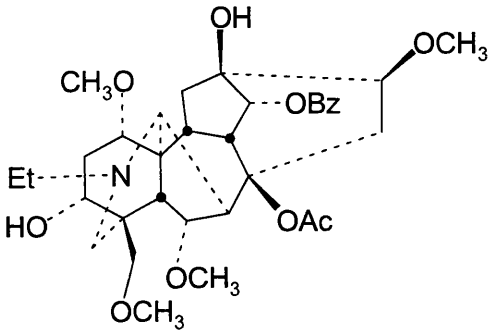

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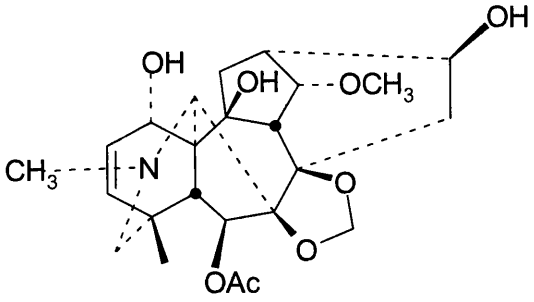

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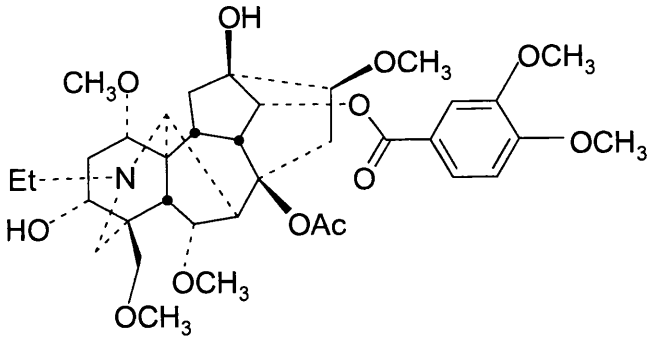

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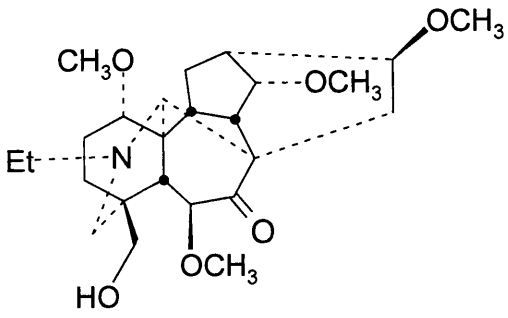

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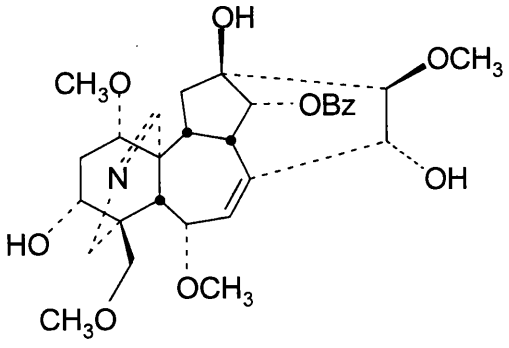

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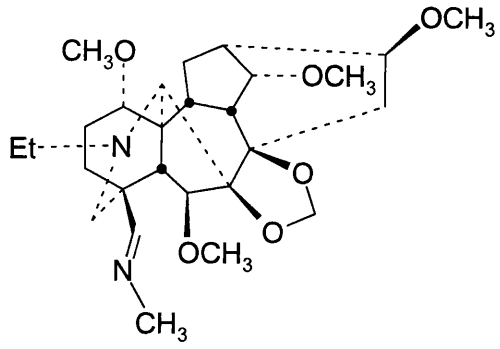

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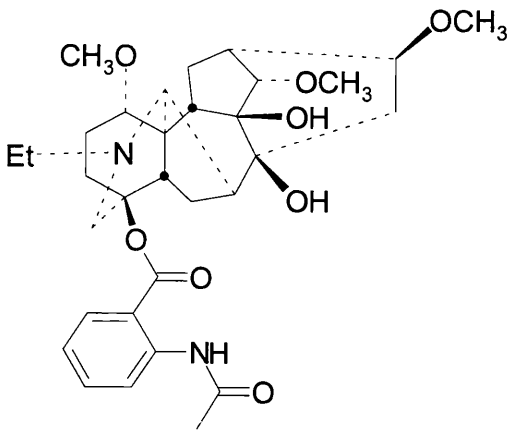

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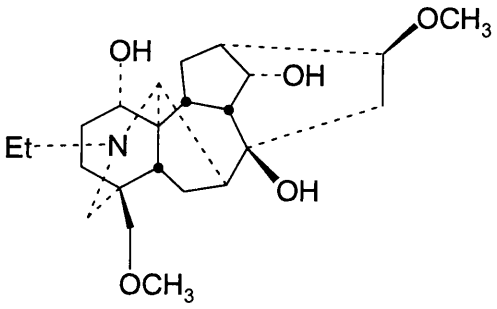

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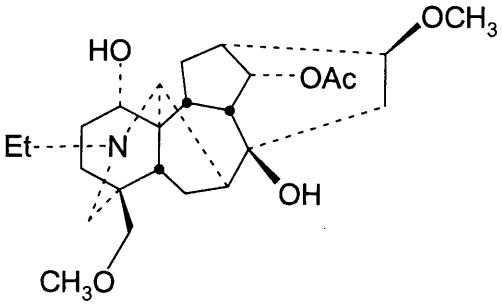

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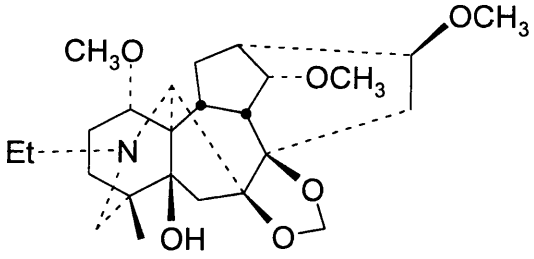

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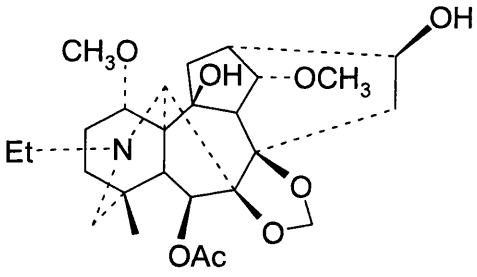

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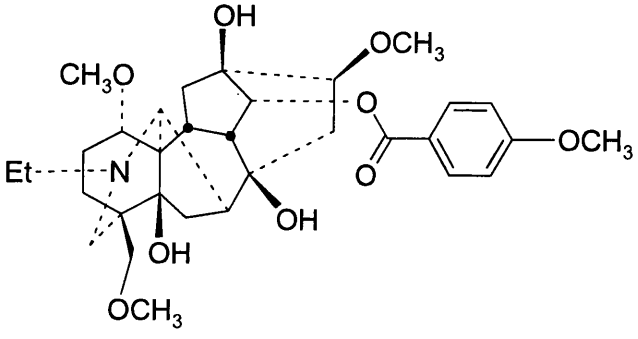


164

Barbeline **141** from *D. barbeyi* Huth is an alkaloid containing a C(19)=N- azomethine group. Pubescenine **142** from plants of *C. pubescens* is the first lycoctonine-type norditerpenoid alkaloid bearing the substitution of C-6 on the α-face. Secojesaconitine **143** and subcumine **145** were isolated from the roots of *A. japonicum* Thunb. Secojesaconitine **143** is the first example of an epoxy ring between C-3 and C-17. The diacetate of 10-hydroxytalatizidine **144** was synthesised from 10-hydroxytalatizidine to assign its structure. Delvestine **146** was isolated from *D. vestitum* Wall. Delsoline **147** was first isolated from *D. consolida* and later identified in many *Aconitum* and *Delphinium* species. Akirine **148** from the epigeal part of *A. kirinensi* Nakai is the first diterpenoid alkaloid with a lycoctonine skeleton containing a 9, 14-methylenedioxy group and a β-oriented substituent at C-14. 1-*epi*-Delphisine **149** has not been found to occur naturally, but has been prepared by the reduction of 1-oxodelphisine. 6-*O*-Deacetylperegrine **150** was synthesized from peregrine, which was isolated from *D. peregrinum* var. *elongatum* Boiss., to confirm the structure of peregrine as suitable crystals from peregrine were not obtained. Chasmanthinine **151**, chasmaconitine **152**, 14-*O*-benzoyl-8-ethoxybikhaconine **153**, 14-*O*-benzoyl-8-methoxybikhaconine **154**, 3α-bikhaconine **155**, and indaconitine **156** were isolated from the roots of *A. chasmanthum* Stapf ex Holmes of Pakistani origin. The pharmacological properties of chasmaconitine **152** included the control and induction of cardiac arrhythmia, effected on smooth and skeleton muscles, central nervous activity, and analgesia. Pseudaconitine **157** was isolated from the roots of *A. falconeri*. Siwanine D **158** was isolated from the aerial parts of *D. siwanense* var. *leptogen* (H.-M.) W.T. Wang. Anhydrolycoctonine **159** was prepared by the alkaline hydrolysis of anhydrolycaconitine which was isolated from the roots of *A. septentrionale* K. Its skeleton differs from usual norditerpenoid alkaloids by the replacement of the C-7 and C-17 bridge by the C-8 and C-17 fragment. Secokaraconitine **160** was isolated from tubers of *A. karacolicum*. Elatidal methylimine **161** was synthesised to study its configuration. Lappaconitine **26** has been used as both a pharmacological and a chromatographic standard. 20-*N*-Ethyl-1,8,14-trihydroxy-16*β*,18-dimethoxylycoctonine **162**, known as talatisidine, was isolated from *D. roylei* Munz. Hemsleyadine **163** was isolated from the roots of *A. hemsleyanum* var. *circinacum*. Nordhagenine A **84** and nordhagenine B **85** were isolated from the aerial parts of *D. nordhgenii* collected in Pakistan. 14-Acetyl-20-ethyl-1,8-dihydroxy-16,18-dimethoxy-lycoctonine **164** has also been studied. These norditerpenoid alkaloids were studied recently by X-ray crystallography and the results are reported as shown and referenced in Table 4. We conclude that the conformations of the rings affect the biological activities only slightly, but the substitution and the position have significantly greater biological effects.

3.5.1 Aconitine

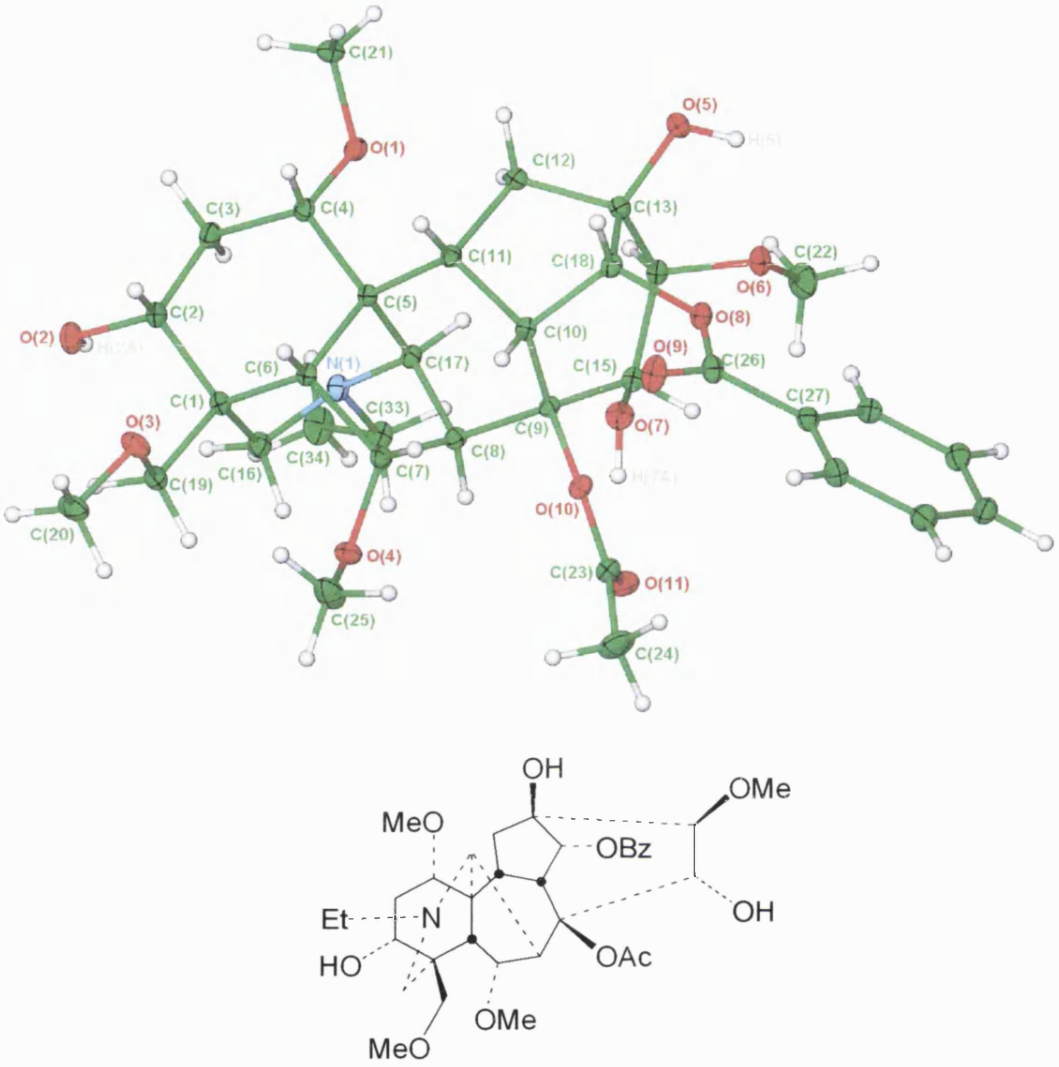


Figure 3.19 Structure of aconitine by X-ray crystallographic analysis

X-Ray crystallographic data for aconitine indicated that the orthorhombic crystal belonged to the space group $P2_12_12_1$. Figure 3.19 shows a composite stereoview of aconitine showing the asymmetric unit, but different atom labelling is used. All hydrogen atoms have been shown.

Aconitine, known since 1833, is one of the most accessible and highly complicated norditerpenoid alkaloids. The structure elucidation of this alkaloid based on chemical degradation studies was independently supported by X-ray crystallographic analysis of demethanolaconione.[173] This investigation not only confirmed the position of the methoxyl and hydroxyl groups in ring A, but also provided the absolute configurations of 13 out of the

15 asymmetric centres of aconitine. Weisner and co-workers showed that the methoxyl group at C-1 of delphinine has an α-equatorial configuration by an X-ray crystal structure determination of the acid oxalate salt of a degradation product obtained from delphinine.[174] As delphinine was correlated earlier with aconitine, the configuration of C-1 of aconitine was established as shown in **12**. The X-ray cryatallographic investigations of other researchers have studied aconitine and related alkaloids.[151] Furthermore, the X-ray crystallography of other norditerpenoid alkaloids have been reviewed.[175]

In our investigations, the relative stereochemistry of the alkaloid at all the centres can be assumed to be correct. The substitution pattern for aconitine can be drawn as C(1)-α-OCH_3, C(3)-α-OH, C(6)- α-OCH_3, C(8)-β-OAc, C(13)-β-OH, C(14)-α-OBz, C(15)-α-OH, C(16)-β-OCH_3, N-CH_2CH_3 and the absolute configuration considered as: 1*S*, 3*R*, *4S*, 5*R*, 6*S*, 8*S*, 9*R*, 10*R*, 11*S*, 13*R*, 14*S*, 15*S*, 16*S*, and 17*R*.

The central ring system of a norditerpenoid alkaloid is traditionally viewed as being three six-membered (two cyclohexane and one piperidine), two five-membered, and one seven-membered fused rings, but could be viewed as being formed by the fusion of four six-membered and two five-membered rings. This inflexible framework only has conformational freedom in ring A and in the free edge of ring D. The ring conformation appears to be determined by H-bond formation. The A/B ring junction is *trans* and all the other ring junctions (A/E, B/C, B/D, and B/F) are *cis*.

Ring A, one of the cyclohexane rings, -C(1)-C(2)-C(3)-C(4)-C(5)-C(11)-, was found to be a chair, with the C-1 substituent, C(1)-α-OCH_3, on the same side of the ring as the N bridge. The chair form for ring A has only been found when no opportunity for formation of a hydrogen bond with the *N* atom exists. This occurs when there is no hydrogen atom donor (a protonated N atom and oxygenated function are on opposite sides of the ring), when there is no oxygenated function at C-1, and when there is hydrogen bond formation with a counter ion.[151, 176] Thus, the chair conformer is expected for aconitine and is often found for lycoctonine-type and aconitine-type free bases, with α-methoxy group at C-1. In the chair conformer, the N atom is exposed and this may effect the interaction with the receptor site.[151] In neutral condition, to form a hydrogen bond the only H-atom donor to the unprotonated *N*-atom is the hydroxyl group on C-3; however, the distance of the nitrogen atom to the oxygen atom at C-3 is 3.945 Å.[151] At biological pH, however, the N atom of such alkaloids may be protonated[151], permitting the formation of a hydrogen bond from the ammonium hydrogen to the oxygen atom of the group at C-1 and thus, stabilising the boat

form (C-2 located *cis* rather that *trans* to C-5 with reference to the plane passing through C-1, C-3, C-4, and C-11).[151]

A boat conformer is often found for this six-membered ring in the salts of norditerpenoid alkaloids (for example, the perchlorate of browniine[76]) due to a hydrogen bond between the N atom and the counter ion.[151] In addition, norditerpenoid alkaloids bearing a C(1)-α-OH, such as delphinifoline, usually exist with ring A in a boat conformation to facilitate the intramolecular hydrogen bonding between the N atom and hydroxyl group with the an unprotonated N atom interacting with hydroxyl hydrogen.[157] Kerr and Codding suggested that the energy barrier between boat and chair forms is relatively low for ring A in these compounds.[176]

The six-membered ring D, -C(8)-C(9)-C(14)-C(13)-C(16)-C(15)-, does not have the flexibility of ring A. It is in boat form with C-15 forming the end atom. The substitution at C-15 can form an intramolecular hydrogen bond between the carbonyl oxygen atom of the acetyl group on C-8 and the hydrogen atom of the hydroxyl group on C-15 (the distance between both atoms is 2.083 Å). Formation of this hydrogen bond and reduction of unfavourable contacts among the substituents on ring D has the net effect of flattening the boat form at C-15. Close contacts between C-15 and the axial substituent on C-14, benzoyl group, may well contribute to the flattening of ring D.[151]

The ring -C(7)-C(8)-C(9)-C(10)-C(11)-C(17)- in aconitine is a distorted chair with C-9 below and C-17 above the plane through the atoms C-7, C-8, C-10, and C-11) whereas, in acoforestine, the atoms C-7 and C-10 deviate from the plane.[157] The piperidine ring, ring E, -C(4)-C(5)-C(11)-C(17)-N-C(19)-, is also a distorted chair, with N above and C-5 below the plane through the atoms C-4, C-11, C-17, and C-19. Five-membered ring C, -C(9)-C(10)-C(12)-C(13)-C(14)-, is in an envelope conformation, with C-14 at the flap. The seven-membered ring B, -C(5)-C(6)-C(7)-C(8)-C(9)-C(10)-C(11)-, is a boat. The five-membered ring F,-C(5)-C(6)-C(7)-C(17)-C(11)-, is an envelope, with C-11 at the flap.

Acetylation of C-15 or deacetylation of C-8 greatly reduces the toxicity of aconitine. Thus, the positions of these hydrogen bond donors and acceptors are likely important in receptor binding. Another intramolecular hydrogen bond is present between the methoxy oxygen atom on C-16 and the hydrogen of the hydroxyl group on C-13.

3.5.2. Mesaconitine

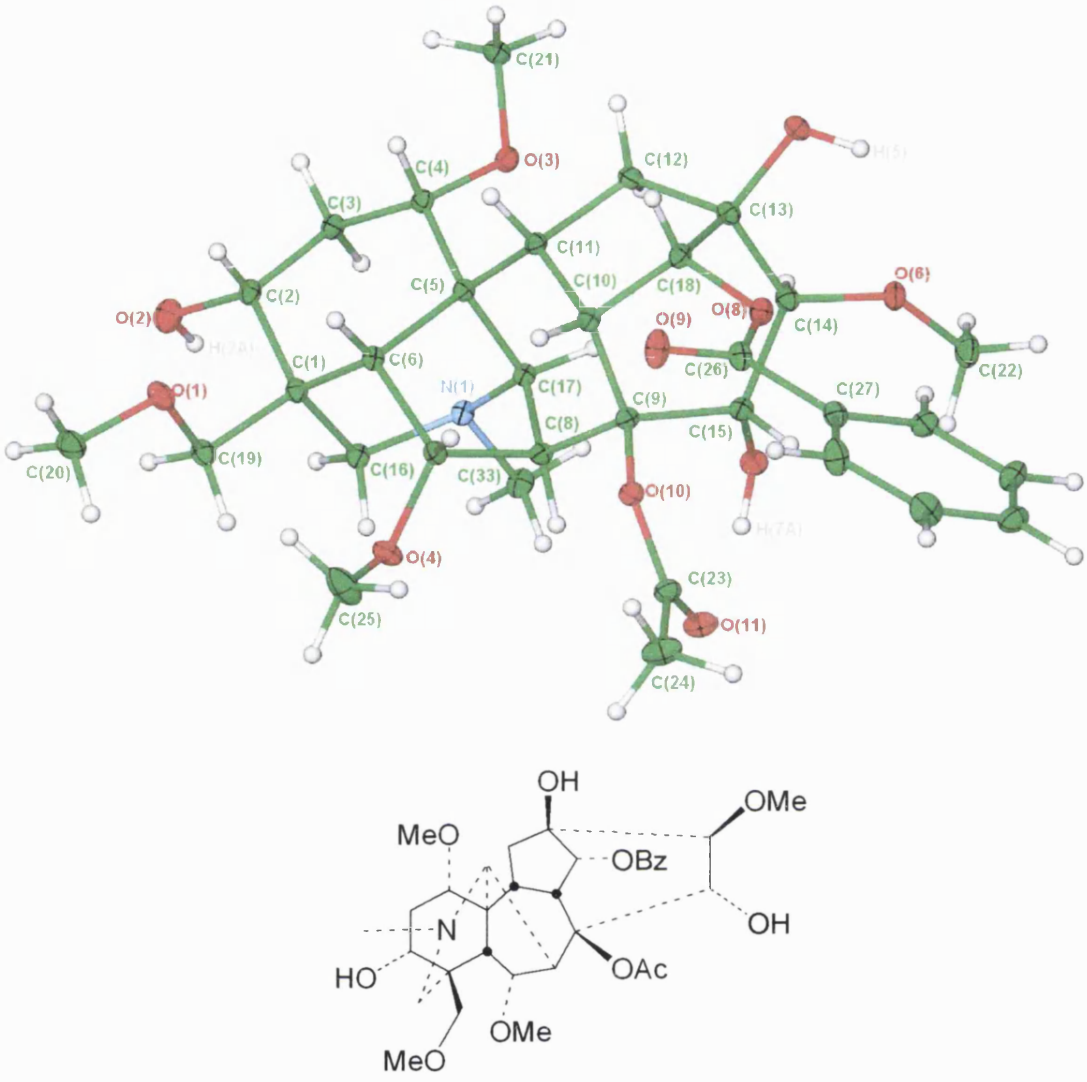


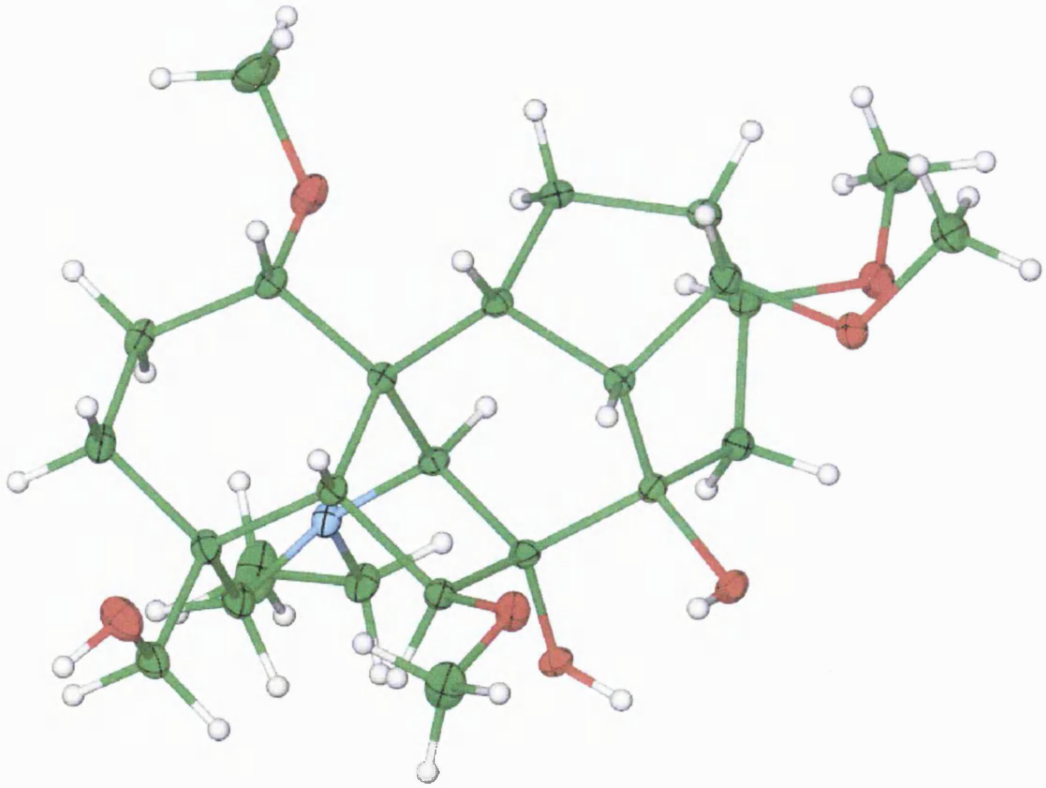
Figure 3.20 Structure of mesaconitine by X-ray crystallographic analysis

X-Ray crystallographic data for mesaconitine indicated that the orthorhombic crystal belonged to the space group $P2_12_12_1$. Figure 3.20 shows a composite stereoview of mesaconitine showing the asymmetric unit, along with the labelling scheme used. All hydrogen atoms have been shown.

In our investigations, the relative stereochemistry of the alkaloid at all the centres can be assumed to be correct. The substitution pattern for mesaconitine can be drawn as C(1)-α-OCH_3, C(3)-α-OH, C(6)- α-OCH_3, C(8)-β-OAc, C(13)-β-OH, C(14)-α-OBz, C(15)-α-OH, C(16)-β-OCH_3, N-CH_3 and the absolute configuration considered as: 1*S*, 3*R*, 4*S*, 5*R*, 6*S*, 8*S*, 9*R*, 10*R*, 11*S*, 13*R*, 14*S*, 15*S*, 16*S*, and 17*R*. The A/B ring junction is *trans* and all the other ring junctions (A/E, B/C, B/D, and B/F) are *cis*.

Mesaconitine differs from aconitine at methyl group attached to the *N*-atom. Thus, the conformations of the rings of mesaconitine are similar to those of aconitine. The conformation of ring A, -C(1)-C(2)-C(3)-C(4)-C(5)-C(11)-, is a chair, but at biological pH, the N atom of this alkaloid may be protonated,[151] changing to the boat form (C-2 located *cis* rather that *trans* to C-5 with reference to the plane passing through C-1, C-3, C-4, and C-11).[151] The seven-membered ring B, -C(5)-C(6)-C(7)-C(8)-C(9)-C(10)-C(11)-, is a boat. Five-membered ring C, -C(9)-C(10)-C(12)-C(13)-C(14)-, is in an envelope conformation, with C-14 at the flap. The six-membered ring D, -C(8)-C(9)-C(14)-C(13)-C(16)-C(15)-, is a boat form with C-15 forming the end atom.. The piperidine ring, ring E, -C(4)-C(5)-C(11)-C(17)-N-C(19)-, is also a distorted chair, with N above and C-5 below the plane through the atoms C-4, C-11, C-17, and C-19. The five-membered ring F,-C(5)-C(6)-C(7)-C(17)-C(11)-, is an envelope, with C-11 at the flap. For some alkaloids, it is possible to describe this ring as a half-chair with an approximate two-fold axis through C-6.

3.5.3. Lycoctonine



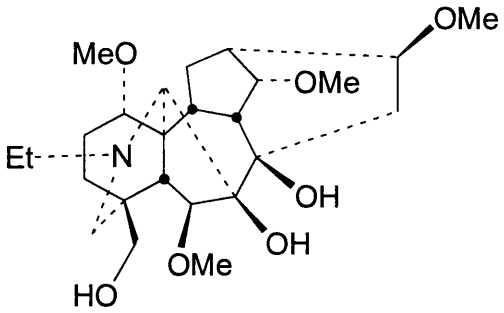


Figure 3.21 Structure of lycoctonine by X-ray crystallographic analysis

X-Ray crystallographic data for lycoctonine indicated that the orthorhombic crystal belonged to the space group $P2_1$. Figure 3.21 shows a composite stereoview of lycoctonine showing the asymmetric unit. All hydrogen atoms have been shown.

In our investigations, the relative stereochemistry of the alkaloid at all the centres can be assumed to be correct. The literature structure of lycoctonine was studied by X-ray analysis of its salt derivative, (+)-des-(oxymethylene)lycoctonine hydroiodide monohydrate.[177] The methoxy or hydroxyl group at C-1 was considered to be in the β-configuration. Edwards and Przybylska revised the stereochemistry for all alkaloids related to lycoctonine with a methoxy group at C-1, such that the original β-assignment was established as an error.[150] The substitution pattern for lycoctonine can be drawn as C(1)-α-OCH_3, C(6)-β-OH, C(7)-β-OH, C(8)- β-OH, C(14)-α-OCH_3, C(16)-β-OCH_3, N-CH_2CH_3 and the absolute configuration considered as: 1*S*, 4*S*, 5*R*, 6*S*, 7*S*, 8*S*, 9*R*, 10*R*, 11*S*, 13*R*, 14*S*, 16*S*, and 17*R*. The A/B ring junction is *trans* and all the other ring junctions (A/E, B/C, B/D, and B/F) are *cis*.

The cyclohexane ring A, -C(1)-C(2)-C(3)-C(4)-C(5)-C(11)-, is a chair, with the C-1 substituent, C(1)-α-OCH_3, on the same side of the ring as the *N* bridge. Ring A is chair because there is no intramolecular hydrogen bond. Like aconitine and mesaconitine, the ring A of lycoctonine can adopt a boat form when the *N* atom protonated, may affecting the biological activities, binding the nAChR.

The seven-membered ring B, -C(5)-C(6)-C(7)-C(8)-C(9)-C(10)-C(11)-, is a boat. Five-membered ring C, -C(9)-C(10)-C(12)-C(13)-C(14)-, is in an envelope conformation, with C-14 at the flap. The six-membered ring D, -C(8)-C(9)-C(14)-C(13)-C(16)-C(15)-, is a half-chair form with C-14 and C-15 forming the end atoms above the plane through C-8, C-9, C-13, and C-16. The piperidine ring, ring E, -C(4)-C(5)-C(11)-C(17)-N-C(19)-, is a chair, with

N above and C-5 below the plane through the atoms C-4, C-11, C-17, and C-19. The five-membered ring F,-C(5)-C(6)-C(7)-C(17)-C(11)-, is an envelope, with C-11 at the flap.

Unlike aconitine, in lycoctonine the H-atom of hydroxyl group at C-8 form an intramolecular hydrogen bond with the methoxy O atom at C-6 (the distance between both atoms is 1.995 Å) and the hydroxyl O atom at C-8 form an intramolecular hydrogen bond with the H-atom of hydroxyl group at C-7 (the distance between both atoms is 2.275 Å). Formation of these hydrogen bonds may have the effect of flattening the boat at C-15. Close contacts between C-15 and the axial methoxy group at C-14 may contribute to the flattening of ring D.

3.5.4. Delpheline

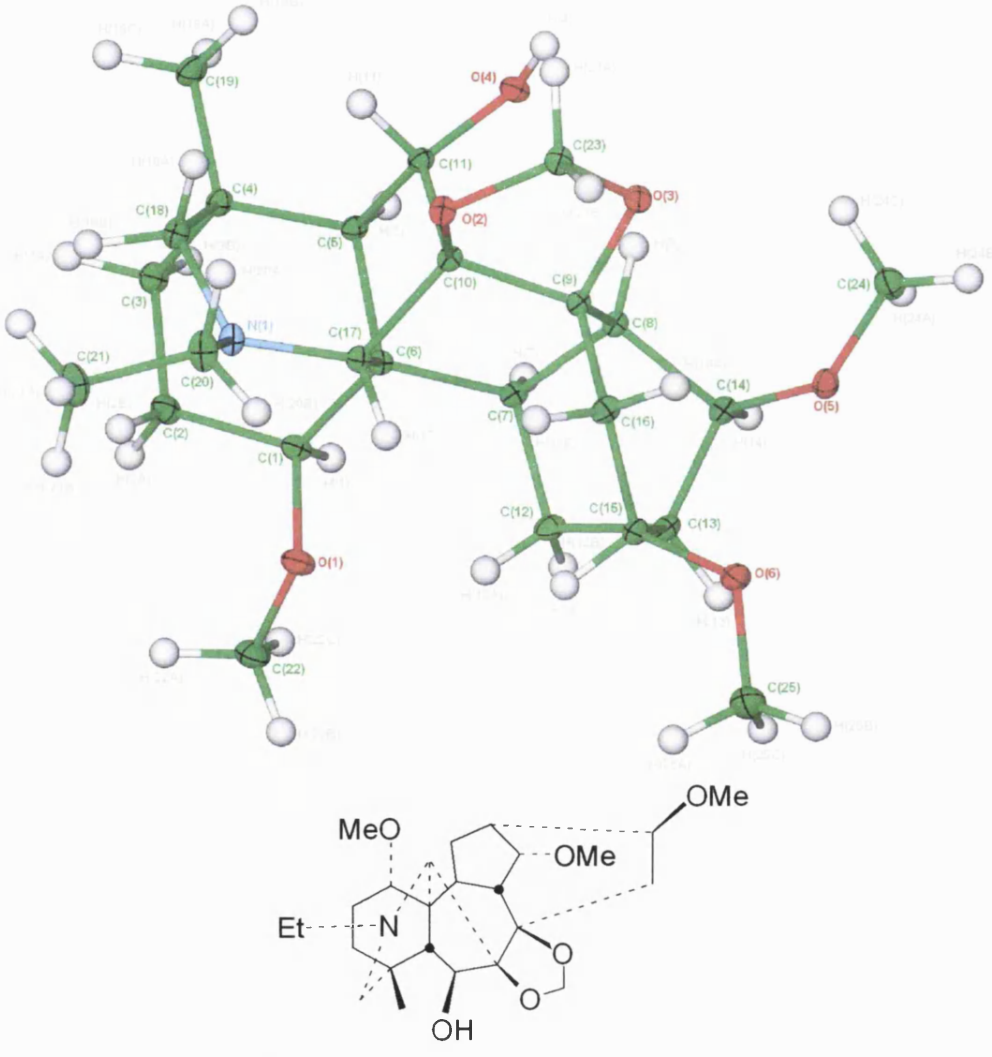


Figure 3.22 Structure of delpheline by X-ray crystallographic analysis

X-Ray crystallographic data for delpheline indicated that the orthorhombic crystal belonged to the space group $P2_12_12_1$. Figure 3.22 shows a composite stereoview of delpheline showing the asymmetric unit, along with the labelling scheme used. All hydrogen atoms have been shown. Therefore, the isolation of the norditerpenoid alkaloid delpheline is confirmed and its characterisation is complete.

In our investigations, the relative stereochemistry of the alkaloid at all the centres can be assumed to be correct. Delpheline is a lycoctonine-type alkaloid so the methoxy group at C-1 is reassigned to be α-configuration.[150] The substitution pattern for delpheline can be drawn as C(1)-α-OCH_3, C(6)-β-OH, C(7)-β-OCH_2O-β-C(8), C(14)-α-OCH_3, C(16)-β-OCH_3, N-CH_2CH_3 and the absolute configuration considered as: 1*S*, 4*S*, 5*R*, 6*S*, 7*S*, 8*S*, 9*R*, 10*R*, 11*S*, 13*R*, 14*S*, 16*S*, and 17*R*.

The carbon skeleton of delpheline is an inflexible framework only has conformational freedom in ring A and in the free edge of ring D. The ring conformation appears to be determined by H-bond formation. The A/B ring junction is *trans* and all the other ring junctions (A/E, B/C, B/D, and B/F) are *cis*.

Ring A, one of the cyclohexane rings, -C(1)-C(2)-C(3)-C(4)-C(5)-C(11)-, was found to be a chair, with the C-1 substituent, C(1)-α-OCH_3, on the same side of the ring as the N bridge. The chair form for ring A has only been found when no opportunity for formation of a hydrogen bond with the N atom exists. This occurs when there is no hydrogen atom donor (a protonated N atom and oxygenated function are on opposite sides of the ring), when there is no oxygenated function at C-1, and when there is hydrogen bond formation with a counter ion.[151, 176] Thus, the chair conformer is expected for delpheline and is often found for lycoctonine-type and aconitine-type free bases, with α-methoxy group at C-1.

In the chair conformer, the N atom is exposed and this may effect the interaction with the receptor site.[151] At biological pH, however, the N atom of such alkaloids may be protonated[151], permitting the formation of a hydrogen bond from the ammonium hydrogen to the oxygen atom of the group at C-1 and thus, stabilising the boat form (C-2 located *cis* rather that *trans* to C-5 with reference to the plane passing through C-1, C-3, C-4, and C-11).[151] A boat form is often found for this six-membered ring in the salts of norditerpenoid alkaloids (for example, the perchlorate of browniine[76]) due to a hydrogen bond between the N atom and the counter ion.[151] In addition, norditerpenoid alkaloids bearing a C(1)-α-OH, such as delphinifoline, usually exist with ring A in a boat conformation to facilitate the

intramolecular hydrogen bonding between the N atom and hydroxyl group with the an unprotonated N atom interacting with hydroxyl hydrogen.[157] Kerr and Codding suggested that the energy barrier between boat and chair forms is relatively low for ring A in these compounds.[176]

The six-membered ring D, -C(8)-C(9)-C(14)-C(13)-C(16)-C(15)-, does not have the flexibility of ring A. It is in half-chair form with C-14 and C-15 forming the end atoms above the plane through C-8, C-9, C-13, and C-16. For alkaloids possessing C-7, C-8 dihydroxy substitution, as in many other lycoctonine-type norditerpenoid alkaloids, a boat (with the end at C-15 flattened) is possible, stabilised by a bifurcated intramolecular hydrogen bond between the hydrogen of C(7)-β-OH and the oxygen of C(8)-β-OH.[176]

The ring -C(7)-C(8)-C(9)-C(10)-C(11)-C(17)- in delpheline is a distorted chair with C-9 below and C-17 above the plane through the atoms C-7, C-8, C-10, and C-11) whereas, in acoforestine, the atoms C-7 and C-10 deviate from the plane.[157] The piperidine ring, ring E, -C(4)-C(5)-C(11)-C(17)-N-C(19)-, is also a distorted chair, with N above and C-5 below the plane through the atoms C-4, C-11, C-17, and C-19. Five-membered ring C, -C(9)-C(10)-C(12)-C(13)-C(14)-, is in an envelope conformation, with C-14 at the flap. The seven-membered ring B, -C(5)-C(6)-C(7)-C(8)-C(9)-C(10)-C(11)-, is a chair, with the C-10 and C-6 atoms deviating from the plane, as seen for aconitine.[151] The five-membered ring F,-C(5)-C(6)-C(7)-C(17)-C(11)-, is puckered. For some alkaloids, it is possible to describe this ring as a half-chair with an approximate two-fold axis through C-6. As an illustration of the effect of the substituents around the norditerpenoid alkaloid skeleton, delcosine which differs from delpheline in that it has hydroxyl groups at C-1, C-7, C-8, and C-14 and methoxy groups at C-6, C-16, and C-18, has rings A, B, and D in boat conformations, rings C and F in envelope form, and ring E as a chair.[76]

Data to be found in Appendix 1, for all four single-crystals (aconitine, mesaconitine, lycoctonine, and delpheline) include: crystal data and structure refinement, atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3) with U(eq) is defined as one third of the trace of the orthogonalized Uij tensor, bond lengths [Å] and angles [°], anisotropic displacement parameters (Å^2 x 10^3) and the anisotropic displacement factor exponent takes the form: -2 gpi^2 [h^2 a*^{2} U11 + ... + 2 h k a* b* U, and hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^2 x 10^3).

3.6. pK_a measurement

$$pH = pK_a + \log\frac{HA}{A^-} \qquad \text{(Eq. 1)}$$

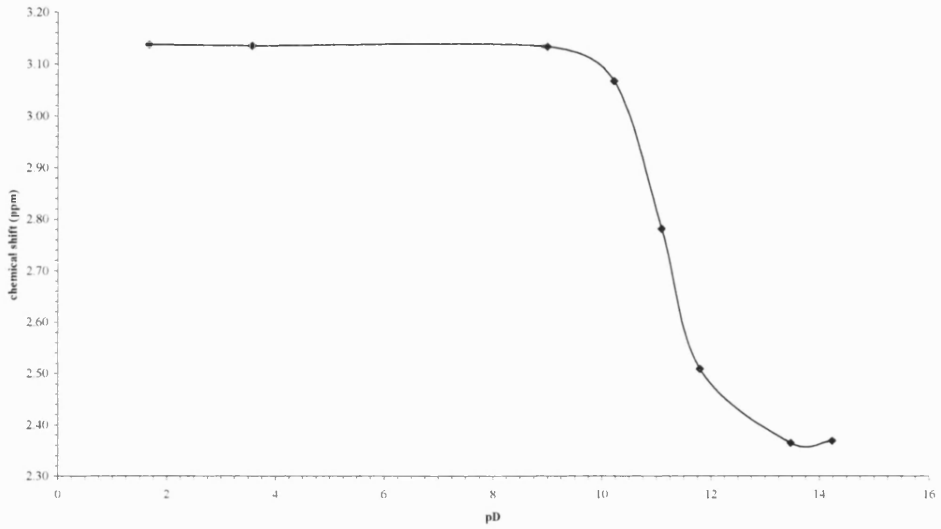
From the Henderson-Hasselbalch equation (Eq. 1), the pH is equal to pK_a when the acid is 50% dissociated. The pK_a value of a compound influence many characteristics of the compound such as its reactivity, and spectral properties (colour) and solubility. In biochemistry the pK_a values of proteins and amino acid side chains are of major importance for the activity of enzymes and the stability of proteins. This property is of general importance in chemistry because ionisation of a compound alters its physical behavior and macro properties such as solubility and lipophilicity. For example, ionisation of any compound will increase the solubility in water, but decrease the lipophilicity. This can be exploited in drug development to increase the concentration of a compound in the blood by adjusting the pK_a of an ionisable group. However, this must be done with caution as an ionised compound will pass less easily through cell membranes.

The traditional method for pK_a measurement is a direct titration. Both indicators and electrodes are used to indicate the end point, but potentiometric titration is more accurate than volumetric titration. However, this method has limitations. First, the reaction between the substance to be determined and the reagent should occur with great speed. This condition is satisfied by most reactions used in acid-base titration. Second, the substance to be determined should react stoichiometrically with the reagent, and no side-reactions should occur. Third, other substances present in the solution should not react or interfere with the main reaction. Last, an indicator should be available for the detection of the end point. If no suitable indicator is available, the end point very often can be found by the application of physico-chemical methods (for example, conductometric titration, amperometric titration and spectrophotometric titration). However, one of the practical difficulties of the pK_a measurement of alkaloids is that alkaloids are weak bases, causing difficult to pinpoint the end points. Other difficulties are their solubility (many alkaloids are soluble in organic solvents, but not freely soluble in aqueous solution) and quite large amounts needed. Owing to the limitation of traditional method for pK_a measurement, there are many physico-chemical properties that can be used to monitor the acid/base reaction. In our studies, the method to measure pK_a is using proton NMR to monitor the reaction. The environment around the protonated nitrogen atom differs from one around the neutral nitrogen atom, including the environment of groups attached to the nitrogen atom.

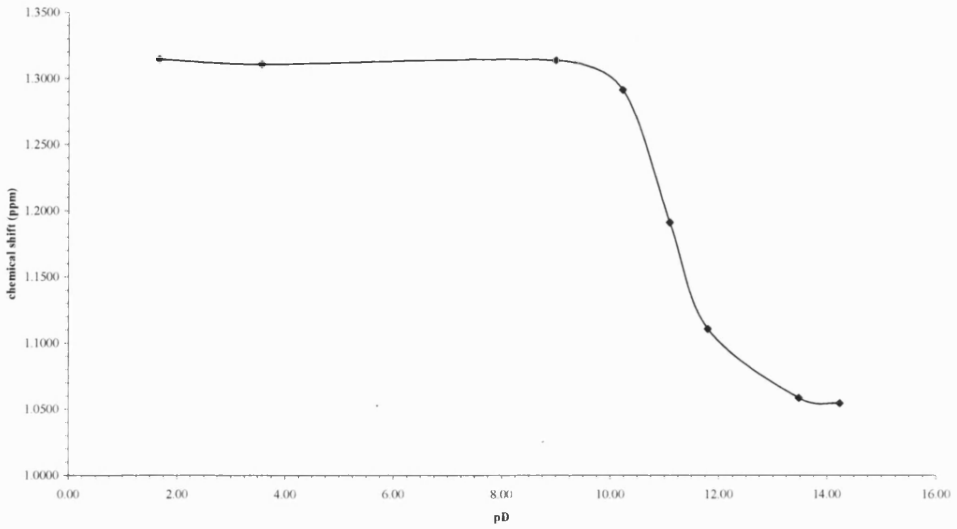
$$pD = pH + 0.4 \qquad \text{(Eq. 2)}$$

The pK_a values of MLA and *N*-ethylpiperidine from graphs of pD vs chemical shift (δ) (see Figure 3.23) were 7.55 and 11.15 respectively. However, Glasore[178] showed the relationship of pD and pH (Eq. 2). pD is the pH value measuring from deuteriated solvent, especially D_2O, and pH is the real value which was obtained from non-deuteriated solvent, then the pK_a of *N*-ethylpiperidine and MLA calculated from Eq. 2 were 10.75 and 7.15, respectively, but the literature pK_a of *N*-ethylpiperidine is 10.45.[179] The pK_a value difference, 0.30 unit, is possibly because the solvent used in the ^{1}H-NMR experiment was deuteriated (D_2O:CD_3OD) while the solvent used in the experiment reported in the literature was 50% aq. ethanol.

Monitoring methylene in ethyl group attached to *N*-atom of *N*-ethylpiperidine



Monitoring methyl in ethyl group attached to *N*-atom of *N*-ethylpiperidine



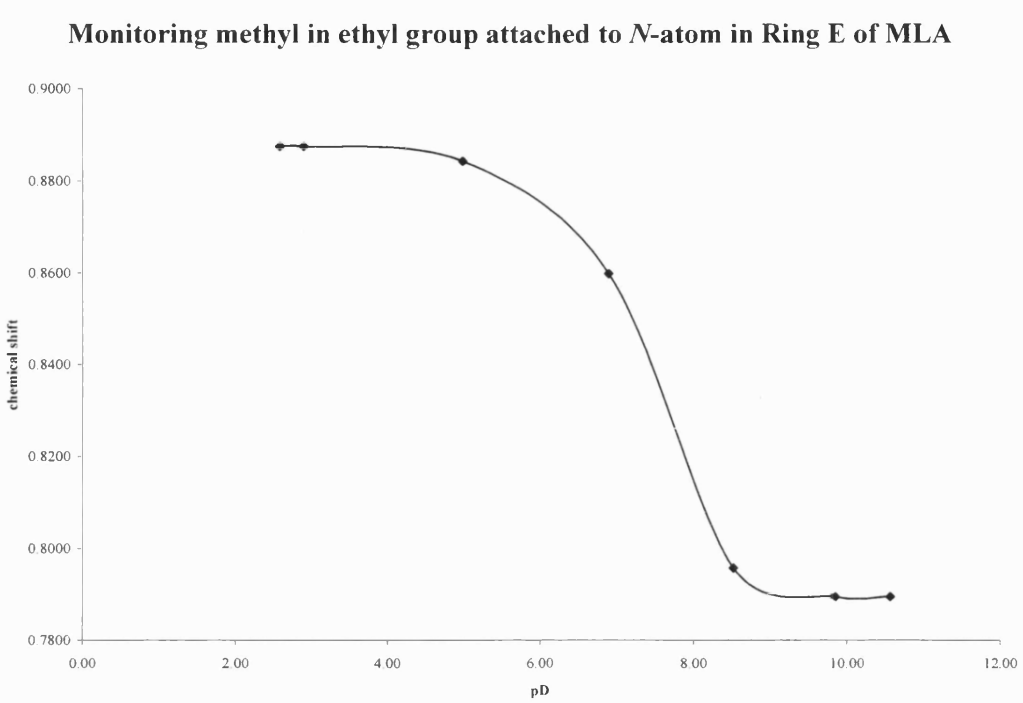
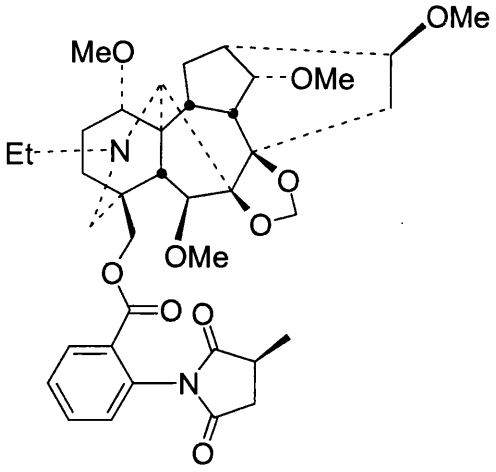


Figure 3.23 Graphs of pD vs δ_H showing: a) *N*-ethylpiperidine monitoring methylene in ethyl group; b) *N*-ethylpiperidine monitoring methyl in ethyl group (*N*-ethylpiperidine was dissolved in $CD_3OD:D_2O$ 3:2); c) MLA monitoring methyl in ethyl group (MLA was dissolved in $CD_3OD:D_2O$ 2:1).

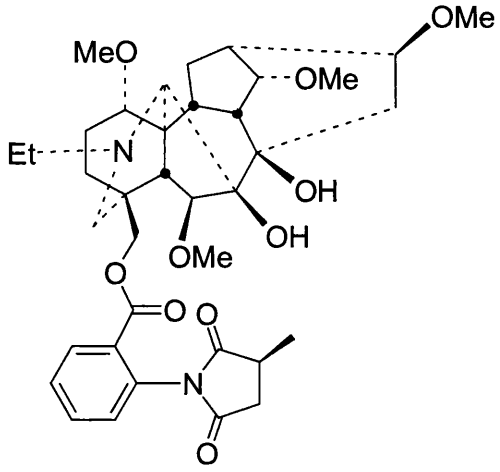
In our experiments, the pK_a values obtained from the monitoring of methylene group and methyl group in ethyl group attached to the *N* atom is slightly different (Figure 3.23) due to the impact of the positive charge of the protonated nitrogen atom depending on the distance. To measure the pK_a value of MLA, three groups (methylene in ethyl group, methylene at C-19, and methine at C-17) are directly attached to the *N* atom, but they are each difficult to monitor in crowded regions of the spectrum, so the methyl in the ethyl group was observed.

This technique has recently been used for measuring pK_a values with only a small amount of compound.[180] Testa and co-workers measured the pK_a and log *P* (partition coefficient) of cetirizine by NMR-pH titration in DMSO:D_2O (8:20).[181] Noszál and co-workers recently reported pK_a values of imatinib (Gleevec, a new signal transduction inhibitor drug of chronic myeloid leukaemia) and its fragments by NMR-pH titration in H_2O.[182] Szakács and Hägele determined accurately the low pK_a of protonated histidine and dichloroacetic acid in $H_2O:D_2O$ (9:1).[183] Like traditional pH-titration protocols, this method has the problem of measuring pH values very exactly. The reported values are often for acids, and the pK_a value of a traditional alkaloid has not yet been reported using this procedure.

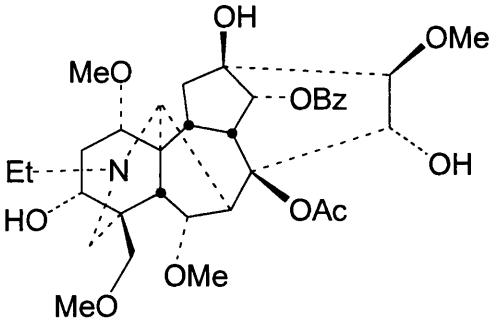
In 1968, Golkiewicz and co-workers measured the pK_a values of selected norditerpenoid alkaloids (aconitine, elatine, and lycoctonine) by a traditional pH-titration method in 80% aqueous methylcellosolve (2-methoxy-ethanol).[184] The corresponding *N*-ethyl nitrogen atoms in piperidine containing polycyclic norditerpenoid alkaloids were found to be ~1000-fold weaker bases than *N*-ethylpiperidine (pK_a = 10.45). Thus, substitution with secondary alcohol and/or *O*-methyl ether functional groups acts to reduce the basicity significantly.



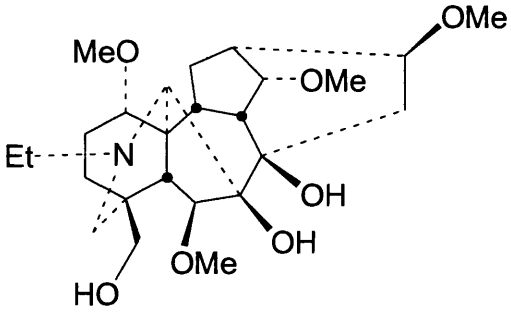
elatine (pK_a= 5.33)[184]



MLA (pK_a = 7.15)



aconitine (pK_a = 7.45)[184]



lycoctonine (pK_a = 7.55)[184]

The pK_a of aconitine is slightly less than that of lycoctonine because aconitine has two strong electron withdrawing groups (*O*-benzoyl and *O*-acetyl groups). Furthermore, MLA is a weaker base than both aconitine and lycoctonine possibly because MLA contains a stronger electron withdrawing group (*O*-2-(methylsuccinimido)benzoyl group). However, in 1968, Golkiewicz and co-workers reported elatine to be a significantly (~100-fold) weaker base than MLA,[184] even though their structures are similar except for the substituent at C-7-C-8 (elatine: methylenedioxy acetal group cf MLA: two hydroxyl groups). This will not be the only error in the norditerpenoid alkaloid literature! Thus, we have shown that the NMR method does provide a way to obtain the valuable pK_a data of alkaloids (MLA pK_a = 7.15), but it does require homogeneous samples.

Conclusions

Plants in the genera *Aconitum*, *Consolida*, and *Delphinium* are important sources of norditerpenoid alkaloids. Some of these alkaloids are highly toxic to mammals. Therefore, their selective modes of action are of interest to biological and medicinal chemists and to pharmacologists as these natural products, their derivatives and analogues may be potent ligands and potentially promising leads for novel selective antagonists and/or agonists of sub-types of nicotinic acetylcholine receptors (nAChR) and voltage-gated sodium channels. This thesis is focussed on the chemistry of norditerpenoid alkaloids from these three genera, starting with a review of compounds recently isolated and not reviewed elsewhere and including brief aspects of taxonomy, biological activities, and modes of action. Selected aspects of the uses in traditional medicine of *Delphinium* and *Aconitum* are presented, including data on both toxicity and detoxification. In this thesis, chemical constituents of the seeds of Delphinium cultivar Pacific Giant and the seeds of *Aconitum lycoctonum* have been investigated.

Crude alkaloidal extracts were obtained using solvents of differing polarity and the crude extracts were purified by repeated column chromatography (over silica and alumina gels), yielding five known norditerpenoid alkaloids from Delphinium cv Pacific Giant (delavaines A and B, delpheline, methyllycaconitine (MLA), and pacinine) and two others from *A. lycoctonum* (lycaconitine and *N*-succinylanthranoyl lycoctonine). The organic extracts were subjected to acid/base cycles, basified to different pH levels (4.6, 7.1, and 10.0). These showed MLA (by TLC) in each pH fraction. Attempts to use vacuum liquid chromatography (VLC) to separate the alkaloids from the crude extract of Delphinium cv Pacific Giant have not been reported previously, and they were unsuccessful.

Delavaines A and B were first isolated from *D. delavayi* Franch var. *pogonanthum* (H.M.) Wang. They are methyl esters which have been considered in the literature to be artefacts arising from the use of methanol as an extracting solvent or as an eluent in silica gel chromatography. However, in this study delavaines were extracted with ethanol and no ethyl esters by succinimido ring-opening were obtained as determined by NMR spectroscopy. Further, a sample of the crude ethanol extract was purified by flash column chromatography over neutral alumina gel using 2-propanol as the alcoholic component of the mobile phase. TLC (cyclohexane:chloroform:diethylamine (5:4:1) detection by Dragendorff spray) and under UV light showed it to be homogeneous ($R_f = 0.15$). 2-Propanyl esters were not shown by NMR spectroscopy or by mass spectrometry and it co-

chromatographed by TLC with authentic methyl ester Delavaines A and B. We therefore concluded that methyl ester Delavaines A and B are natural products and not artefacts of isolation.

Delpheline was first isolated from the seeds of *D. elatum* L. in 1943 by a traditional extraction process and also from *D. barbeyi*. In our studies, delpheline was isolated (for the first time) from Delphinium cv Pacific Giant which was selected and hybridised from *D. elatum*. The X-ray structure (not yet in a public database) of delpheline was established from a recrystallized sample obtained from the crude hexane extract.

Like delpheline, MLA was first isolated from the seeds of *D. elatum* L. in 1943 and it has subsequently isolated from many *Delphinium* species and also *Inula royleana*. In our studies, MLA was isolated from Delphinium cv Pacific Giant purified by flash column chromatography over neutral alumina gel.

Pacinine, a 6-keto oxidation product of delpheline, was first isolated from the seeds of Delphinium cv Pacific Giant in 1989 and was also obtained in our studies.

Lycaconitine, the parent member of the MLA series, was first isolated from *A. lycoctonum* in 1884 and it was also isolated from *A. gigas*, *A. umbrosum* and *D. cashmirianum*. In our studies, lycaconitine was isolated from the crude ethanol extract of *A. lycoctonum* seeds.

N-Succinylanthranoyl lycoctonine was first isolated from *A. gigas* in 1978 and also from *A. barbatum* var. *puberulum* in 1982. It is the first time *N*-succinylanthranoyl lycoctonine has been reported from the crude ethanolic extract of *A. lycoctonum* seeds.

X-Ray crystallographic analysis of aconitine, mesaconitine, lycoctonine, and delpheline was also studied. The X-ray structures of aconitine, mesaconitine, and lycoctonine have been published with the main emphasis on structure elucidation. They are useful to compare with the X-ray structure of delpheline in the conformation of the rings, the relative configuration of the chiral centres, and their relationship to biological activities.

Three compounds were obtained from semi-synthesis starting with MLA: lycoctonine by basic hydrolysis (saponification), inuline by acidic hydrolysis and by esterification of lycoctonine, and elatine by methylenedioxy acetal formation.

Lycoctonine is a natural product first isolated from *A. lycoctonum* in 1884, but in our studies lycoctonine was mainly obtained from the basic hydrolysis of (semi-purified) MLA. Inuline or anthranoyllycoctonine is a natural product isolated from *Inula royleana* and many *Delphinium* species. In our studies, inuline was obtained from acidic hydrolysis of MLA, but proved difficult to separate from (residual) MLA efficiently, so inuline was obtained from the esterification of lycoctonine with isatoic anhydride. Though this was only achieved in low yield (12%), it proved easier to purify from the residual starting material and by-products. Elatine is a natural product first isolated from *D. elatum*, but in our studies elatine was obtained from the methylenedioxy acetal formation of MLA even though it is in low yield (7%) despite three attempts. This conversion of MLA into elatine requires the two tertiary alcohols (at C7 and C8) to act as nucleophiles and this was not efficient.

MLA is a selective competitive antagonist at α7 sub-type nAChR. From collaborative studies, the biological activities for methyl esters delavaines A and B (a 3:2 mixture), delpheline, and pacinine (the B-ring C6-ketone of delpheline) are reported in this thesis. Their biological activities were determined in competitive α-bungarotoxin binding assays for α7 nAChR in rat brain membranes. Delavaines A and B were potent ligands (IC_{50} = 50 nM, cf MLA IC_{50} = ~1-2 nM), whereas the closely related delpheline (C6 secondary alcohol) and pacinine (C6 ketone) displayed modest activity at α7 nAChR (IC_{50} = ~1 μM).

The measurement of pK_a (a key physico-chemical parameter) can be challenging on small samples of natural products. A ^{1}H NMR spectroscopic technique has been used to determine the pK_a values for proteins and amino acids, but in this study it was used for measuring the pK_a of alkaloids. *N*-Ethylpiperidine is a model alkaloid for the norditerpenoids in its class which are the focus of this thesis. Its pK_a measured by ^{1}H NMR is 10.75, but its literature pK_a value is 10.45. The pK_a value difference is possibly because the solvent used in the ^{1}H-NMR experiment was deuteriated (D_2O:CD_3OD 2:3) while the solvent used in the literature experiment was 50% aq. ethanol. The pK_a of MLA determined by this method was 7.15.

We achieved the aims of our research with the isolation and purification of norditerpenoid alkaloids from Delphinium cv Pacific Giant and *Aconitum lycoctonum* seeds followed by characterisation chromatographically, spectroscopically, and by X-ray analysis. Semi-syntheses of three norditerpenoid alkaloids, lycoctonine, inuline, and elatine were achieved. A novel NMR approach to determine the key physico-chemical parameter, pK_a, was demonstrated as a proof of the applicability of such a spectroscopic technique for alkaloids.

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Appendix 1

Data from four single crystal X-ray determinations:

Tables 1.1-1.5 Crystal data and structure refinement for aconitine.

Tables 2.1-2.5 Crystal data and structure refinement for mesaconitine.

Tables 3.1-3.5 Crystal data and structure refinement for lycoctonine.

Tables 4.1-4.5 Crystal data and structure refinement for delpheline.

Table 1.1 Crystal data and structure refinement for aconitine.

Identification code	k04farm5
Empirical formula	$C_{34} H_{47} N O_{11}$
Formula weight	645.73
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 12.4580(1)Å $\alpha = 90^\circ$
	b = 15.3360(2)Å $\beta = 90^\circ$
	c = 16.6870(3)Å $\gamma = 90^\circ$
Volume	3188.15(8) $Å^3$
Z	4
Density (calculated)	1.345 Mg/m^3
Absorption coefficient	0.100 mm^{-1}
F(000)	1384
Crystal size	0.35 x 0.30 x 0.25 mm
Theta range for data collection	3.74 to 30.05°
Index ranges	-17<=h<=17; -20<=k<=21; -23<=l<=23
Reflections collected	31552
Independent reflections	9317 [R(int) = 0.0455]
Reflections observed (>2σ)	7641
Data Completeness	0.996
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.97 and 0.92
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	9317 / 0 / 424
Goodness-of-fit on F^2	1.014
Final R indices [I>2σ(I)]	$R^1 = 0.0428$ $wR_2 = 0.0897$
R indices (all data)	$R^1 = 0.0602$ $wR_2 = 0.0975$
Absolute structure parameter	0.6(5)
Largest diff. peak and hole	0.288 and -0.205 $eÅ^{-3}$

Notes: Intermolecular and intramolecular hydrogen bonding present.

Hydrogen bonds with H..A < r(A) + 2.000 Angstroms and <DHA > 110 deg.

D-H	d(D-H)	d(H..A)	<DHA	d(D..A)	A
O2-H2A	0.840				

Alternative approximate positions for H attached to O2: NONE

O5-H5	0.840	2.070	117.68	2.570	O6
O7-H7A	0.840	2.082	138.51	2.768	O11

Table 1.2 Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for aconitine. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)
O(1)	8352(1)	8356(1)	5967(1)	24(1)
O(2)	4638(1)	8885(1)	5439(1)	36(1)
O(3)	5102(1)	9325(1)	3428(1)	33(1)
O(4)	6808(1)	11120(1)	3661(1)	31(1)
O(5)	11947(1)	8843(1)	4855(1)	30(1)
O(6)	12161(1)	10468(1)	5196(1)	30(1)
O(7)	10006(1)	11484(1)	5462(1)	31(1)
O(8)	11514(1)	10151(1)	3547(1)	25(1)
O(9)	10931(1)	10037(1)	2271(1)	36(1)
O(10)	9538(1)	11260(1)	3494(1)	24(1)
O(11)	9470(1)	12537(1)	4175(1)	36(1)
N(1)	7161(1)	10361(1)	5731(1)	24(1)
C(1)	6002(1)	9696(1)	4653(1)	24(1)
C(2)	5673(1)	8829(1)	5058(1)	27(1)
C(3)	6501(1)	8514(1)	5650(1)	26(1)
C(4)	7633(1)	8482(1)	5307(1)	21(1)
C(5)	7983(1)	9286(1)	4809(1)	19(1)
C(6)	7083(1)	9566(1)	4200(1)	21(1)
C(7)	7542(1)	10445(1)	3871(1)	23(1)
C(8)	8313(1)	10776(1)	4536(1)	21(1)
C(9)	9465(1)	10698(1)	4219(1)	20(1)
C(10)	9603(1)	9781(1)	3856(1)	20(1)
C(11)	9077(1)	9063(1)	4390(1)	20(1)
C(12)	10017(1)	8813(1)	4974(1)	24(1)
C(13)	11004(1)	9336(1)	4718(1)	23(1)
C(14)	11053(1)	10214(1)	5181(1)	23(1)
C(15)	10382(1)	10960(1)	4813(1)	22(1)
C(16)	6151(1)	10434(1)	5270(1)	26(1)
C(17)	8116(1)	10155(1)	5253(1)	21(1)
C(18)	10775(1)	9501(1)	3837(1)	23(1)
C(19)	5083(1)	9932(1)	4076(1)	27(1)
C(20)	4262(1)	9473(1)	2871(1)	40(1)
C(21)	8495(1)	7467(1)	6172(1)	30(1)
C(22)	12442(2)	11033(1)	5837(1)	45(1)
C(23)	9451(1)	12132(1)	3554(1)	30(1)
C(24)	9310(2)	12530(1)	2741(1)	46(1)
C(25)	6548(2)	11106(2)	2823(1)	46(1)
C(26)	11495(1)	10379(1)	2767(1)	25(1)
C(27)	12279(1)	11094(1)	2615(1)	23(1)
C(28)	12881(1)	11461(1)	3232(1)	26(1)
C(29)	13618(1)	12115(1)	3062(1)	30(1)
C(30)	13752(1)	12400(1)	2282(1)	33(1)
C(31)	13170(1)	12025(1)	1666(1)	33(1)
C(32)	12434(1)	11375(1)	1827(1)	29(1)
C(33)	7322(1)	11127(1)	6236(1)	29(1)
C(34)	6633(2)	11101(1)	6978(1)	44(1)

Table 1.3 Bond lengths [Å] and angles [°] for aconitine.

O(1)-C(21)	1.4171(18)	O(1)-C(4)	1.4319(17)
O(2)-C(2)	1.4401(17)	O(3)-C(20)	1.4183(19)
O(3)-C(19)	1.4269(19)	O(4)-C(7)	1.4259(18)
O(4)-C(25)	1.435(2)	O(5)-C(13)	1.4162(17)
O(6)-C(22)	1.421(2)	O(6)-C(14)	1.4346(17)
O(7)-C(15)	1.4272(17)	O(8)-C(26)	1.3477(18)
O(8)-C(18)	1.4407(17)	O(9)-C(26)	1.2057(18)
O(10)-C(23)	1.3455(19)	O(10)-C(9)	1.4884(17)
O(11)-C(23)	1.208(2)	N(1)-C(33)	1.460(2)
N(1)-C(17)	1.4664(18)	N(1)-C(16)	1.4784(19)
C(1)-C(19)	1.539(2)	C(1)-C(16)	1.542(2)
C(1)-C(2)	1.547(2)	C(1)-C(6)	1.5574(19)
C(2)-C(3)	1.508(2)	C(3)-C(4)	1.523(2)
C(4)-C(5)	1.551(2)	C(5)-C(17)	1.534(2)
C(5)-C(11)	1.5689(18)	C(5)-C(6)	1.572(2)
C(6)-C(7)	1.564(2)	C(7)-C(8)	1.554(2)
C(8)-C(9)	1.5343(19)	C(8)-C(17)	1.5488(19)
C(9)-C(10)	1.5414(19)	C(9)-C(15)	1.565(2)
C(10)-C(18)	1.5220(19)	C(10)-C(11)	1.562(2)
C(11)-C(12)	1.571(2)	C(12)-C(13)	1.5285(19)
C(13)-C(18)	1.518(2)	C(13)-C(14)	1.553(2)
C(14)-C(15)	1.544(2)	C(23)-C(24)	1.498(2)
C(26)-C(27)	1.490(2)	C(27)-C(28)	1.392(2)
C(27)-C(32)	1.398(2)	C(28)-C(29)	1.388(2)
C(29)-C(30)	1.384(2)	C(30)-C(31)	1.382(3)
C(31)-C(32)	1.380(2)	C(33)-C(34)	1.507(2)
C(21)-O(1)-C(4)	113.20(12)	C(20)-O(3)-C(19)	112.34(12)
C(7)-O(4)-C(25)	111.88(13)	C(22)-O(6)-C(14)	114.54(13)
C(26)-O(8)-C(18)	119.53(11)	C(23)-O(10)-C(9)	120.63(12)
C(33)-N(1)-C(17)	112.01(11)	C(33)-N(1)-C(16)	110.87(12)
C(17)-N(1)-C(16)	115.03(12)	C(19)-C(1)-C(16)	109.61(12)
C(19)-C(1)-C(2)	106.16(12)	C(16)-C(1)-C(2)	111.80(13)
C(19)-C(1)-C(6)	111.69(12)	C(16)-C(1)-C(6)	108.28(11)
C(2)-C(1)-C(6)	109.32(11)	O(2)-C(2)-C(3)	110.01(12)
O(2)-C(2)-C(1)	112.24(12)	C(3)-C(2)-C(1)	112.32(12)
C(2)-C(3)-C(4)	113.45(12)	O(1)-C(4)-C(3)	107.14(12)
O(1)-C(4)-C(5)	110.09(11)	C(3)-C(4)-C(5)	115.81(12)
C(17)-C(5)-C(4)	117.53(12)	C(17)-C(5)-C(11)	108.12(10)
C(4)-C(5)-C(11)	107.99(11)	C(17)-C(5)-C(6)	98.79(11)
C(4)-C(5)-C(6)	111.29(11)	C(11)-C(5)-C(6)	113.04(11)
C(1)-C(6)-C(7)	112.08(11)	C(1)-C(6)-C(5)	109.77(11)
C(7)-C(6)-C(5)	101.60(11)	O(4)-C(7)-C(8)	109.55(12)
O(4)-C(7)-C(6)	118.55(12)	C(8)-C(7)-C(6)	104.91(11)
C(9)-C(8)-C(17)	111.57(11)	C(9)-C(8)-C(7)	107.82(11)
C(17)-C(8)-C(7)	104.62(11)	O(10)-C(9)-C(8)	107.01(11)
O(10)-C(9)-C(10)	101.65(10)	C(8)-C(9)-C(10)	108.13(11)
O(10)-C(9)-C(15)	108.84(11)	C(8)-C(9)-C(15)	116.33(11)
C(10)-C(9)-C(15)	113.68(11)	C(18)-C(10)-C(9)	111.87(11)
C(18)-C(10)-C(11)	102.43(11)	C(9)-C(10)-C(11)	111.83(11)

C(10)-C(11)-C(5)	117.72(11)	C(10)-C(11)-C(12)	102.27(11)
C(5)-C(11)-C(12)	115.19(12)	C(13)-C(12)-C(11)	107.42(11)
O(5)-C(13)-C(18)	113.65(12)	O(5)-C(13)-C(12)	110.02(12)
C(18)-C(13)-C(12)	101.91(11)	O(5)-C(13)-C(14)	110.51(12)
C(18)-C(13)-C(14)	110.14(12)	C(12)-C(13)-C(14)	110.32(12)
O(6)-C(14)-C(15)	109.08(12)	O(6)-C(14)-C(13)	106.37(11)
C(15)-C(14)-C(13)	115.07(12)	O(7)-C(15)-C(14)	107.13(12)
O(7)-C(15)-C(9)	112.67(11)	C(14)-C(15)-C(9)	117.20(11)
N(1)-C(16)-C(1)	113.23(12)	N(1)-C(17)-C(5)	111.22(11)
N(1)-C(17)-C(8)	114.66(12)	C(5)-C(17)-C(8)	100.23(11)
O(8)-C(18)-C(13)	108.69(12)	O(8)-C(18)-C(10)	115.08(12)
C(13)-C(18)-C(10)	102.01(11)	O(3)-C(19)-C(1)	107.99(12)
O(11)-C(23)-O(10)	124.98(15)	O(11)-C(23)-C(24)	124.78(15)
O(10)-C(23)-C(24)	110.24(15)	O(9)-C(26)-O(8)	124.09(14)
O(9)-C(26)-C(27)	125.80(14)	O(8)-C(26)-C(27)	110.10(12)
C(28)-C(27)-C(32)	119.72(14)	C(28)-C(27)-C(26)	121.69(14)
C(32)-C(27)-C(26)	118.55(14)	C(29)-C(28)-C(27)	119.83(15)
C(30)-C(29)-C(28)	120.03(15)	C(31)-C(30)-C(29)	120.27(15)
C(32)-C(31)-C(30)	120.28(16)	C(31)-C(32)-C(27)	119.85(15)
N(1)-C(33)-C(34)	111.97(14)		

Symmetry transformations used to generate equivalent atoms.

Table 1.4 Anisotropic displacement parameters ($Å^2 \times 10^3$) for aconitine. The anisotropic displacement factor exponent takes the form: $-2\ gpi^2\ [\ h^2\ a^{*2}\ U11 + ... + 2\ h\ k\ a^*\ b^*\ U$

Atom	U11	U22	U33	U23	U13	U12
O(1)	26(1)	25(1)	23(1)	4(1)	-4(1)	-4(1)
O(2)	23(1)	45(1)	39(1)	3(1)	7(1)	-5(1)
O(3)	29(1)	34(1)	35(1)	-5(1)	-13(1)	8(1)
O(4)	28(1)	30(1)	34(1)	9(1)	-2(1)	4(1)
O(5)	20(1)	30(1)	39(1)	2(1)	1(1)	2(1)
O(6)	22(1)	36(1)	33(1)	-2(1)	-3(1)	-7(1)
O(7)	34(1)	29(1)	29(1)	-8(1)	2(1)	0(1)
O(8)	22(1)	29(1)	23(1)	-2(1)	4(1)	-4(1)
O(9)	36(1)	49(1)	25(1)	-8(1)	4(1)	-16(1)
O(10)	27(1)	25(1)	22(1)	4(1)	3(1)	-4(1)
O(11)	43(1)	24(1)	41(1)	0(1)	1(1)	2(1)
N(1)	22(1)	28(1)	22(1)	-4(1)	5(1)	-2(1)
C(1)	19(1)	26(1)	26(1)	0(1)	1(1)	1(1)
C(2)	20(1)	29(1)	31(1)	2(1)	2(1)	-3(1)
C(3)	24(1)	28(1)	26(1)	2(1)	3(1)	-4(1)
C(4)	21(1)	23(1)	20(1)	1(1)	-2(1)	-4(1)
C(5)	18(1)	21(1)	18(1)	0(1)	1(1)	-1(1)
C(6)	20(1)	22(1)	21(1)	0(1)	1(1)	-2(1)
C(7)	21(1)	25(1)	23(1)	3(1)	1(1)	0(1)
C(8)	22(1)	19(1)	21(1)	0(1)	3(1)	0(1)
C(9)	21(1)	20(1)	18(1)	2(1)	3(1)	-2(1)
C(10)	20(1)	22(1)	18(1)	-3(1)	2(1)	-3(1)
C(11)	20(1)	19(1)	21(1)	-2(1)	2(1)	-1(1)
C(12)	22(1)	23(1)	28(1)	3(1)	1(1)	1(1)
C(13)	19(1)	23(1)	26(1)	1(1)	2(1)	1(1)
C(14)	22(1)	28(1)	21(1)	-1(1)	0(1)	-4(1)
C(15)	22(1)	23(1)	21(1)	-4(1)	2(1)	-2(1)
C(16)	23(1)	26(1)	28(1)	-3(1)	5(1)	1(1)
C(17)	21(1)	21(1)	20(1)	0(1)	4(1)	-2(1)
C(18)	22(1)	22(1)	24(1)	-3(1)	4(1)	-3(1)
C(19)	21(1)	28(1)	31(1)	-1(1)	-1(1)	2(1)
C(20)	36(1)	36(1)	47(1)	-1(1)	-19(1)	5(1)
C(21)	31(1)	27(1)	34(1)	5(1)	-4(1)	3(1)
C(22)	38(1)	48(1)	49(1)	-14(1)	-13(1)	-9(1)
C(23)	27(1)	25(1)	37(1)	7(1)	1(1)	-3(1)
C(24)	62(1)	36(1)	41(1)	16(1)	-8(1)	-8(1)
C(25)	35(1)	65(1)	38(1)	22(1)	-6(1)	5(1)
C(26)	21(1)	32(1)	22(1)	-4(1)	5(1)	-2(1)
C(27)	20(1)	26(1)	24(1)	-1(1)	3(1)	1(1)
C(28)	27(1)	26(1)	24(1)	-3(1)	2(1)	1(1)
C(29)	29(1)	27(1)	33(1)	-7(1)	0(1)	-2(1)
C(30)	28(1)	27(1)	42(1)	1(1)	6(1)	-2(1)
C(31)	32(1)	38(1)	30(1)	9(1)	5(1)	0(1)
C(32)	26(1)	38(1)	24(1)	-1(1)	1(1)	-2(1)
C(33)	31(1)	30(1)	26(1)	-6(1)	6(1)	-3(1)
C(34)	44(1)	53(1)	35(1)	-13(1)	17(1)	-3(1)

Table 1.5 Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for aconitine.

Atom	x	y	z	U(eq)
H(2A)	4699	9149	5878	43
H(5)	12474	9181	4903	36
H(7A)	9754	11953	5282	37
H(2)	5617	8378	4626	32
H(3A)	6298	7924	5836	31
H(3B)	6497	8906	6122	31
H(4)	7683	7957	4953	26
H(6)	7006	9126	3761	25
H(1)	7984	10309	3386	28
H(8)	8144	11393	4685	25
H(10)	9293	9763	3303	24
H(11)	8945	8546	4039	24
H(12A)	9821	8957	5533	29
H(12B)	10168	8181	4940	29
H(14)	10807	10113	5743	28
H(15)	10894	11331	4502	26
H(16A)	6143	11001	4987	31
H(16B)	5538	10428	5647	31
H(17)	8765	10135	5605	25
H(18)	10856	8949	3524	27
H(19A)	4386	9901	4358	32
H(19B)	5181	10532	3871	32
H(20A)	3568	9423	3144	59
H(20B)	4302	9039	2441	59
H(20C)	4334	10058	2642	59
H(21A)	7820	7232	6383	46
H(21B)	9057	7417	6581	46
H(21C)	8707	7137	5695	46
H(22A)	12093	11599	5761	68
H(22B)	13222	11111	5848	68
H(22C)	12203	10777	6345	68
H(24A)	9320	13167	2787	69
H(24B)	8622	12343	2514	69
H(24C)	9896	12340	2390	69
H(25A)	7203	11190	2508	69
H(25B)	6038	11574	2703	69
H(25C)	6225	10542	2686	69
H(28)	12788	11265	3767	31
H(29)	14030	12366	3481	36
H(30)	14245	12856	2169	39
H(31)	13278	12214	1130	40
H(32)	12034	11119	1403	35
H(33A)	7149	11658	5925	35
H(33B)	8087	11160	6395	35
H(34A)	6766	11624	7301	66
H(34B)	6811	10581	7293	66
H(34C)	5875	11080	6823	66

Table 2.1 Crystal data and structure refinement for mesaconitine.

Identification code	k04farm6
Empirical formula	$C_{33}H_{45}NO_{11}$
Formula weight	631.70
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 12.6760(2)Å α = 90°
	b = 15.4150(2)Å β = 90°
	c = 15.6000(2)Å γ = 90°
Volume	3048.25(7) $Å^3$
Z	4
Density (calculated)	1.376 Mg/m^3
Absorption coefficient	0.103 mm^{-1}
F(000)	1352
Crystal size	0.50 x 0.40 x 0.20 mm
Theta range for data collection	3.71 to 30.02°
Index ranges	-17<=h<=17; -21<=k<=21; -21<=l<=21
Reflections collected	28040
Independent reflections	8840 [R(int) = 0.0446]
Reflections observed (>2σ)	7862
Data Completeness	0.995
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.97 and 0.92
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	8840 / 0 / 410
Goodness-of-fit on F^2	1.020
Final R indices [I>2σ(I)]	$R^1 = 0.0420$ $wR_2 = 0.1021$
R indices (all data)	$R^1 = 0.0512$ $wR_2 = 0.1076$
Absolute structure parameter	-0.5(6)
Largest diff. peak and hole	0.337 and -0.366 $eÅ^{-3}$

Notes: Intermolecular and intramolecular hydrogen bonding present.

Hydrogen bonds with H..A < r(A) + 2.000 Angstroms and <DHA > 110 deg.

D-H	d(D-H)	d(H..A)	<DHA	d(D..A)	A
O2-H2A	0.840	2.225	162.25	3.036	O9 [-x+2, y-1/2, -z+1/2]
O5-H5	0.840	2.035	120.19	2.562	O6
O7-H7A	0.840	2.118	137.47	2.795	O11

Table 2.2 Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for mesaconitine. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)
O(1)	12330(1)	3960(1)	3057(1)	29(1)
O(2)	12779(1)	1901(1)	3763(1)	33(1)
O(3)	9107(1)	1341(1)	4258(1)	23(1)
O(4)	10679(1)	3464(1)	1306(1)	27(1)
O(5)	5625(1)	2674(1)	3779(1)	27(1)
O(6)	5317(1)	2169(1)	2234(1)	26(1)
O(7)	7397(1)	1627(1)	1252(1)	24(1)
O(8)	6075(1)	3907(1)	2345(1)	22(1)
O(9)	6743(1)	5255(1)	2232(1)	33(1)
O(10)	7950(1)	3755(1)	1202(1)	20(1)
O(11)	7953(1)	2877(1)	36(1)	30(1)
N(1)	10270(1)	1395(1)	2329(1)	20(1)
C(1)	11443(1)	2608(1)	2841(1)	19(1)
C(2)	11746(1)	2286(1)	3745(1)	21(1)
C(3)	10943(1)	1667(1)	4108(1)	21(1)
C(4)	9830(1)	2039(1)	4092(1)	19(1)
C(5)	9497(1)	2513(1)	3257(1)	17(1)
C(6)	10390(1)	3117(1)	2899(1)	19(1)
C(7)	9947(1)	3372(1)	1995(1)	20(1)
C(8)	9144(1)	2645(1)	1772(1)	18(1)
C(9)	8033(1)	3034(1)	1837(1)	18(1)
C(10)	7952(1)	3537(1)	2692(1)	19(1)
C(11)	8455(1)	3016(1)	3447(1)	18(1)
C(12)	7510(1)	2451(1)	3775(1)	23(1)
C(13)	6540(1)	2719(1)	3258(1)	21(1)
C(14)	6410(1)	2137(1)	2458(1)	21(1)
C(15)	7089(1)	2405(1)	1676(1)	20(1)
C(16)	11286(1)	1853(1)	2208(1)	21(1)
C(17)	9338(1)	1938(1)	2465(1)	18(1)
C(18)	6819(1)	3640(1)	2991(1)	20(1)
C(19)	12349(1)	3196(1)	2540(1)	23(1)
C(20)	13156(2)	4540(1)	2853(1)	33(1)
C(21)	8955(1)	1176(1)	5143(1)	28(1)
C(22)	4976(2)	1515(1)	1657(1)	36(1)
C(23)	7928(1)	3591(1)	352(1)	25(1)
C(24)	7861(2)	4419(1)	-144(1)	37(1)
C(25)	10899(2)	4344(1)	1110(2)	44(1)
C(26)	6129(1)	4704(1)	2006(1)	23(1)
C(27)	5317(1)	4801(1)	1326(1)	22(1)
C(28)	4701(1)	4096(1)	1074(1)	25(1)
C(29)	3929(2)	4201(1)	456(1)	31(1)
C(30)	3765(2)	5007(1)	81(1)	32(1)
C(31)	4362(2)	5705(1)	334(1)	36(1)
C(32)	5143(1)	5610(1)	956(1)	32(1)
C(33)	10123(1)	748(1)	1660(1)	26(1)

Table 2.3 Bond lengths [Å] and angles [°] for mesaconitine.

O(1)-C(20)	1.414(2)	O(1)-C(19)	1.4269(19)
O(2)-C(2)	1.4381(18)	O(3)-C(21)	1.4181(19)
O(3)-C(4)	1.4363(18)	O(4)-C(25)	1.418(2)
O(4)-C(7)	1.4279(18)	O(5)-C(13)	1.4181(18)
O(6)-C(22)	1.420(2)	O(6)-C(14)	1.4306(19)
O(7)-C(15)	1.4244(17)	O(8)-C(26)	1.3400(19)
O(8)-C(18)	1.4402(18)	O(9)-C(26)	1.2049(19)
O(10)-C(23)	1.3504(19)	O(10)-C(9)	1.4928(17)
O(11)-C(23)	1.206(2)	N(1)-C(33)	1.4550(19)
N(1)-C(17)	1.4632(18)	N(1)-C(16)	1.4812(19)
C(1)-C(19)	1.537(2)	C(1)-C(16)	1.539(2)
C(1)-C(2)	1.544(2)	C(1)-C(6)	1.5509(19)
C(2)-C(3)	1.506(2)	C(3)-C(4)	1.524(2)
C(4)-C(5)	1.553(2)	C(5)-C(17)	1.5338(19)
C(5)-C(11)	1.560(2)	C(5)-C(6)	1.569(2)
C(6)-C(7)	1.567(2)	C(7)-C(8)	1.555(2)
C(8)-C(9)	1.533(2)	C(8)-C(17)	1.554(2)
C(9)-C(10)	1.5467(19)	C(9)-C(15)	1.561(2)
C(10)-C(18)	1.518(2)	C(10)-C(11)	1.561(2)
C(11)-C(12)	1.568(2)	C(12)-C(13)	1.528(2)
C(13)-C(18)	1.521(2)	C(13)-C(14)	1.546(2)
C(14)-C(15)	1.549(2)	C(23)-C(24)	1.495(2)
C(26)-C(27)	1.486(2)	C(27)-C(32)	1.393(2)
C(27)-C(28)	1.394(2)	C(28)-C(29)	1.384(2)
C(29)-C(30)	1.387(3)	C(30)-C(31)	1.374(3)
C(31)-C(32)	1.394(3)		
C(20)-O(1)-C(19)	112.49(13)	C(21)-O(3)-C(4)	113.36(12)
C(25)-O(4)-C(7)	112.61(13)	C(22)-O(6)-C(14)	115.12(13)
C(26)-O(8)-C(18)	120.35(12)	C(23)-O(10)-C(9)	120.92(11)
C(33)-N(1)-C(17)	113.11(12)	C(33)-N(1)-C(16)	110.29(12)
C(17)-N(1)-C(16)	116.61(11)	C(19)-C(1)-C(16)	110.26(12)
C(19)-C(1)-C(2)	106.40(12)	C(16)-C(1)-C(2)	112.08(12)
C(19)-C(1)-C(6)	111.21(12)	C(16)-C(1)-C(6)	108.03(11)
C(2)-C(1)-C(6)	108.89(12)	O(2)-C(2)-C(3)	110.23(12)
O(2)-C(2)-C(1)	112.15(12)	C(3)-C(2)-C(1)	112.25(12)
C(2)-C(3)-C(4)	112.38(12)	O(3)-C(4)-C(3)	107.79(11)
O(3)-C(4)-C(5)	109.25(11)	C(3)-C(4)-C(5)	116.29(12)
C(17)-C(5)-C(4)	116.12(11)	C(17)-C(5)-C(11)	109.26(11)
C(4)-C(5)-C(11)	107.72(11)	C(17)-C(5)-C(6)	98.67(11)
C(4)-C(5)-C(6)	112.44(11)	C(11)-C(5)-C(6)	112.53(11)
C(1)-C(6)-C(7)	112.53(12)	C(1)-C(6)-C(5)	109.93(11)
C(7)-C(6)-C(5)	102.15(11)	O(4)-C(7)-C(8)	109.16(12)
O(4)-C(7)-C(6)	118.00(12)	C(8)-C(7)-C(6)	104.79(12)
C(9)-C(8)-C(17)	111.93(12)	C(9)-C(8)-C(7)	107.70(11)
C(17)-C(8)-C(7)	104.24(11)	O(10)-C(9)-C(8)	108.21(11)
O(10)-C(9)-C(10)	101.18(10)	C(8)-C(9)-C(10)	108.39(11)
O(10)-C(9)-C(15)	107.48(11)	C(8)-C(9)-C(15)	116.78(11)
C(10)-C(9)-C(15)	113.53(12)	C(18)-C(10)-C(9)	112.37(12)
C(18)-C(10)-C(11)	102.03(11)	C(9)-C(10)-C(11)	111.40(11)

C(5)-C(11)-C(10)	117.27(11)	C(5)-C(11)-C(12)	115.60(12)
C(10)-C(11)-C(12)	102.72(11)	C(13)-C(12)-C(11)	106.98(12)
O(5)-C(13)-C(18)	113.05(12)	O(5)-C(13)-C(12)	110.02(12)
C(18)-C(13)-C(12)	102.08(12)	O(5)-C(13)-C(14)	110.38(12)
C(18)-C(13)-C(14)	110.23(12)	C(12)-C(13)-C(14)	110.83(12)
O(6)-C(14)-C(13)	106.23(12)	O(6)-C(14)-C(15)	109.69(12)
C(13)-C(14)-C(15)	114.96(12)	O(7)-C(15)-C(14)	107.04(12)
O(7)-C(15)-C(9)	112.79(12)	C(14)-C(15)-C(9)	117.69(12)
N(1)-C(16)-C(1)	112.99(12)	N(1)-C(17)-C(5)	109.97(12)
N(1)-C(17)-C(8)	115.37(12)	C(5)-C(17)-C(8)	100.14(11)
O(8)-C(18)-C(10)	115.71(12)	O(8)-C(18)-C(13)	107.80(12)
C(10)-C(18)-C(13)	101.94(11)	O(1)-C(19)-C(1)	107.53(12)
O(11)-C(23)-O(10)	124.92(14)	O(11)-C(23)-C(24)	124.63(15)
O(10)-C(23)-C(24)	110.45(14)	O(9)-C(26)-O(8)	124.30(15)
O(9)-C(26)-C(27)	125.90(15)	O(8)-C(26)-C(27)	109.79(13)
C(32)-C(27)-C(28)	119.56(15)	C(32)-C(27)-C(26)	119.66(14)
C(28)-C(27)-C(26)	120.74(14)	C(29)-C(28)-C(27)	120.05(15)
C(28)-C(29)-C(30)	120.31(17)	C(31)-C(30)-C(29)	119.85(16)
C(30)-C(31)-C(32)	120.60(17)	C(27)-C(32)-C(31)	119.62(16)

Symmetry transformations used to generate equivalent atoms.

Table 2.4 Anisotropic displacement parameters ($Å^2$ x 10^3) for mesaconitine. The anisotropic displacement factor exponent takes the form: -2 gpi^2 [h^2 a^{*2} U11 + ... + 2 h k a* b* U.

Atom	U11	U22	U33	U23	U13	U12
O(1)	27(1)	24(1)	36(1)	-6(1)	9(1)	-8(1)
O(2)	27(1)	33(1)	40(1)	-4(1)	-7(1)	6(1)
O(3)	25(1)	20(1)	22(1)	3(1)	-3(1)	-4(1)
O(4)	26(1)	28(1)	27(1)	7(1)	7(1)	0(1)
O(5)	18(1)	37(1)	25(1)	-3(1)	5(1)	1(1)
O(6)	19(1)	28(1)	30(1)	-5(1)	-2(1)	-3(1)
O(7)	27(1)	21(1)	25(1)	-7(1)	3(1)	0(1)
O(8)	19(1)	19(1)	27(1)	-1(1)	-3(1)	3(1)
O(9)	26(1)	21(1)	52(1)	-4(1)	-12(1)	-1(1)
O(10)	21(1)	20(1)	20(1)	3(1)	-2(1)	2(1)
O(11)	40(1)	28(1)	23(1)	-1(1)	1(1)	3(1)
N(1)	20(1)	18(1)	23(1)	-4(1)	-2(1)	3(1)
C(1)	17(1)	19(1)	22(1)	-2(1)	0(1)	1(1)
C(2)	17(1)	21(1)	23(1)	-2(1)	-3(1)	3(1)
C(3)	20(1)	21(1)	22(1)	1(1)	-4(1)	2(1)
C(4)	19(1)	18(1)	19(1)	-1(1)	-2(1)	-2(1)
C(5)	17(1)	17(1)	18(1)	0(1)	-1(1)	2(1)
C(6)	18(1)	17(1)	21(1)	-1(1)	-1(1)	2(1)
C(7)	19(1)	20(1)	22(1)	1(1)	1(1)	1(1)
C(8)	18(1)	19(1)	18(1)	-1(1)	0(1)	4(1)
C(9)	19(1)	18(1)	18(1)	0(1)	-1(1)	2(1)
C(10)	18(1)	18(1)	21(1)	-3(1)	-1(1)	4(1)
C(11)	17(1)	21(1)	18(1)	-1(1)	-1(1)	3(1)
C(12)	19(1)	28(1)	20(1)	2(1)	3(1)	1(1)
C(13)	17(1)	24(1)	21(1)	-2(1)	3(1)	3(1)
C(14)	19(1)	20(1)	23(1)	-2(1)	1(1)	1(1)
C(15)	20(1)	19(1)	20(1)	-3(1)	0(1)	2(1)
C(16)	19(1)	21(1)	23(1)	-4(1)	0(1)	3(1)
C(17)	18(1)	17(1)	19(1)	-2(1)	-1(1)	2(1)
C(18)	18(1)	22(1)	21(1)	-4(1)	-3(1)	5(1)
C(19)	19(1)	22(1)	28(1)	-3(1)	3(1)	0(1)
C(20)	33(1)	30(1)	35(1)	-1(1)	6(1)	-10(1)
C(21)	28(1)	33(1)	24(1)	4(1)	3(1)	-6(1)
C(22)	27(1)	38(1)	41(1)	-12(1)	-3(1)	-8(1)
C(23)	25(1)	28(1)	20(1)	3(1)	-1(1)	3(1)
C(24)	56(1)	30(1)	27(1)	8(1)	-3(1)	8(1)
C(25)	33(1)	37(1)	62(1)	24(1)	10(1)	0(1)
C(26)	18(1)	18(1)	32(1)	-4(1)	0(1)	3(1)
C(27)	18(1)	22(1)	27(1)	-1(1)	1(1)	3(1)
C(28)	28(1)	23(1)	24(1)	-2(1)	-2(1)	1(1)
C(29)	31(1)	33(1)	28(1)	-7(1)	-7(1)	2(1)
C(30)	29(1)	45(1)	23(1)	-3(1)	-3(1)	11(1)
C(31)	31(1)	35(1)	43(1)	13(1)	-2(1)	8(1)
C(32)	23(1)	24(1)	49(1)	6(1)	-6(1)	1(1)
C(33)	28(1)	21(1)	30(1)	-9(1)	-3(1)	3(1)

Table 2.5 Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for mesaconitine.

Atom	x	y	z	U(eq)
H(2A)	12765	1424	3502	40
H(5)	5105	2530	3477	32
H(7A)	7659	1749	772	29
H(2)	11765	2805	4130	25
H(3A)	11136	1525	4707	25
H(3B)	10957	1122	3773	25
H(4)	9769	2463	4575	23
H(6)	10474	3643	3268	22
H(7)	9551	3930	2055	24
H(8A)	9276	2410	1184	22
H(10)	8296	4118	2638	22
H(11)	8619	3445	3910	22
H(12A)	7662	1827	3689	27
H(12B)	7389	2554	4394	27
H(14)	6594	1527	2618	25
H(15)	6602	2714	1274	23
H(16A)	11320	2081	1615	25
H(16B)	11871	1433	2280	25
H(17)	8696	1568	2535	21
H(18)	6784	4038	3497	24
H(19A)	13033	2894	2605	28
H(19B)	12255	3350	1928	28
H(20A)	13112	5051	3226	49
H(20B)	13093	4721	2253	49
H(20C)	13837	4252	2940	49
H(21A)	8455	697	5214	42
H(21B)	8675	1697	5421	42
H(21C)	9631	1019	5406	42
H(22A)	4221	1586	1541	53
H(22B)	5101	943	1911	53
H(22C)	5371	1564	1119	53
H(24A)	7856	4911	254	56
H(24B)	7212	4423	-484	56
H(24C)	8472	4466	-526	56
H(25A)	11403	4372	634	66
H(25B)	11202	4629	1615	66
H(25C)	10245	4638	946	66
H(28)	4812	3543	1327	30
H(29)	3509	3720	287	37
H(30)	3241	5075	-349	39
H(31)	4241	6258	83	44
H(32)	5555	6096	1127	38
H(33A)	10770	406	1598	40
H(33B)	9963	1039	1117	40
H(33C)	9537	364	1815	40

Table 3.1 Crystal data and structure refinement for lycoctonine.

Identification code	k04farm4
Empirical formula	$C_{25}H_{43}NO_8$
Formula weight	485.60
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P21
Unit cell dimensions	a = 11.1690(2)Å α = 90°
	b = 7.8070(1)Å β = 103.944(1)°
	c = 14.6870(3)Å γ = 90°
Volume	1242.91(4) $Å^3$
Z	2
Density (calculated)	1.298 Mg/m^3
Absorption coefficient	0.096 mm^{-1}
F(000)	528
Crystal size	0.35 x 0.20 x 0.10 mm
Theta range for data collection	3.69 to 27.87°
Index ranges	-14<=h<=14; -10<=k<=10; -19<=l<=19
Reflections collected	23647
Independent reflections	5854 [R(int) = 0.0359]
Reflections observed (>2σ)	5298
Data Completeness	0.995
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5854 / 3 / 324
Goodness-of-fit on F^2	1.018
Final R indices [I>2σ(I)]	R^1 = 0.0394 wR_2 = 0.0995
R indices (all data)	R^1 = 0.0468 wR_2 = 0.1052
Absolute structure parameter	1.0(7) Confirmation of sterochem. by chemist.
Largest diff. peak and hole	0.719 and -0.223 $eÅ^{-3}$

Notes: Intra and intermolecular hydrogen-bonding present. One molecule of water present in the asymmetric unit.

Hydrogen bonds with H..A < r(A) + 2.000 Angstroms and <DHA > 110 deg.

D-H	d(D-H)	d(H..A)	<DHA	d(D..A)	A
O1-H1	0.840	1.870	175.13	2.707	O8 [-x+1, y-1/2, -z+2]
O3-H3	0.840	2.275	114.24	2.729	O4
O4-H4	0.840	1.995	131.69	2.629	O2
O8-H8A	0.909	1.948	179.37	2.857	O5
O8-H8B	0.885	1.964	161.76	2.818	O6 [-x, y-1/2, -z+2]

Table 3.2 Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for lycoctonine. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)
O(1)	5987(1)	920(2)	8477(1)	40(1)
O(2)	3848(1)	4274(2)	9463(1)	30(1)
O(3)	3271(1)	7310(1)	7835(1)	26(1)
O(4)	2238(1)	6777(2)	9317(1)	27(1)
O(5)	-205(1)	5314(2)	9445(1)	30(1)
O(6)	-1479(1)	7376(2)	7546(1)	33(1)
O(7)	867(1)	1826(2)	5824(1)	34(1)
O(8)	1601(1)	5039(2)	11185(1)	43(1)
N(1)	3185(1)	4872(2)	6352(1)	25(1)
C(1)	4515(2)	2559(2)	7320(1)	25(1)
C(2)	4237(2)	965(2)	6701(1)	30(1)
C(3)	2996(2)	1139(2)	6007(1)	28(1)
C(4)	1983(2)	1476(2)	6515(1)	23(1)
C(5)	2234(1)	2900(2)	7284(1)	20(1)
C(6)	3571(2)	2704(2)	7935(1)	22(1)
C(7)	3810(1)	4419(2)	8485(1)	24(1)
C(8)	2784(1)	5655(2)	7950(1)	21(1)
C(9)	1730(1)	5839(2)	8461(1)	21(1)
C(10)	1295(1)	4044(2)	8670(1)	22(1)
C(11)	1205(2)	2777(2)	7841(1)	22(1)
C(12)	-151(2)	3039(2)	7242(1)	28(1)
C(13)	-750(2)	4377(2)	7753(1)	27(1)
C(14)	-41(2)	4078(2)	8767(1)	26(1)
C(15)	681(2)	7022(2)	7934(1)	25(1)
C(16)	-542(2)	6203(2)	7417(1)	26(1)
C(17)	4441(2)	4220(2)	6730(1)	29(1)
C(18)	2348(1)	4761(2)	6980(1)	21(1)
C(19)	3199(2)	6529(2)	5885(1)	30(1)
C(20)	3420(3)	6338(3)	4911(2)	56(1)
C(21)	5851(2)	2414(2)	7910(1)	32(1)
C(22)	4991(2)	3615(3)	10002(2)	42(1)
C(23)	-1439(2)	5245(3)	9590(2)	40(1)
C(24)	-2604(2)	7259(3)	6843(2)	52(1)
C(25)	223(2)	316(3)	5457(2)	49(1)

Table 3.3 Bond lengths [Å] and angles [°] for lycoctonine.

O(1)-C(21)	1.421(2)	O(2)-C(22)	1.426(2)
O(2)-C(7)	1.431(2)	O(3)-C(8)	1.4274(19)
O(4)-C(9)	1.4472(18)	O(5)-C(14)	1.430(2)
O(5)-C(23)	1.445(2)	O(6)-C(24)	1.424(2)
O(6)-C(16)	1.437(2)	O(7)-C(25)	1.418(3)
O(7)-C(4)	1.432(2)	N(1)-C(18)	1.4650(19)
N(1)-C(19)	1.465(2)	N(1)-C(17)	1.469(2)
C(1)-C(2)	1.528(2)	C(1)-C(21)	1.538(2)
C(1)-C(6)	1.549(2)	C(1)-C(17)	1.551(2)
C(2)-C(3)	1.516(3)	C(3)-C(4)	1.521(2)
C(4)-C(5)	1.561(2)	C(5)-C(18)	1.534(2)
C(5)-C(11)	1.567(2)	C(5)-C(6)	1.574(2)
C(6)-C(7)	1.553(2)	C(7)-C(8)	1.558(2)
C(8)-C(9)	1.549(2)	C(8)-C(18)	1.556(2)
C(9)-C(10)	1.538(2)	C(9)-C(15)	1.545(2)
C(10)-C(14)	1.533(2)	C(10)-C(11)	1.553(2)
C(11)-C(12)	1.571(2)	C(12)-C(13)	1.531(2)
C(13)-C(14)	1.527(2)	C(13)-C(16)	1.545(2)
C(15)-C(16)	1.534(2)	C(19)-C(20)	1.517(3)
C(22)-O(2)-C(7)	113.05(14)	C(14)-O(5)-C(23)	111.35(14)
C(24)-O(6)-C(16)	114.01(15)	C(25)-O(7)-C(4)	112.68(14)
C(18)-N(1)-C(19)	115.27(13)	C(18)-N(1)-C(17)	115.97(12)
C(19)-N(1)-C(17)	111.44(14)	C(2)-C(1)-C(21)	107.78(14)
C(2)-C(1)-C(6)	109.71(13)	C(21)-C(1)-C(6)	112.43(14)
C(2)-C(1)-C(17)	111.89(14)	C(21)-C(1)-C(17)	106.86(14)
C(6)-C(1)-C(17)	108.20(13)	C(3)-C(2)-C(1)	110.40(14)
C(2)-C(3)-C(4)	110.71(13)	O(7)-C(4)-C(3)	107.96(13)
O(7)-C(4)-C(5)	110.55(12)	C(3)-C(4)-C(5)	116.87(14)
C(18)-C(5)-C(4)	118.52(12)	C(18)-C(5)-C(11)	109.41(12)
C(4)-C(5)-C(11)	107.90(12)	C(18)-C(5)-C(6)	97.83(12)
C(4)-C(5)-C(6)	110.44(12)	C(11)-C(5)-C(6)	112.59(12)
C(1)-C(6)-C(7)	108.11(13)	C(1)-C(6)-C(5)	109.39(12)
C(7)-C(6)-C(5)	104.55(12)	O(2)-C(7)-C(6)	114.46(13)
O(2)-C(7)-C(8)	113.31(13)	C(6)-C(7)-C(8)	104.97(12)
O(3)-C(8)-C(9)	109.19(12)	O(3)-C(8)-C(18)	110.02(12)
C(9)-C(8)-C(18)	112.24(12)	O(3)-C(8)-C(7)	111.44(13)
C(9)-C(8)-C(7)	111.45(13)	C(18)-C(8)-C(7)	102.39(12)
O(4)-C(9)-C(10)	111.36(12)	O(4)-C(9)-C(15)	102.96(12)
C(10)-C(9)-C(15)	114.02(13)	O(4)-C(9)-C(8)	106.45(12)
C(10)-C(9)-C(8)	109.01(12)	C(15)-C(9)-C(8)	112.69(13)
C(14)-C(10)-C(9)	111.35(13)	C(14)-C(10)-C(11)	101.75(12)
C(9)-C(10)-C(11)	112.80(12)	C(10)-C(11)-C(5)	117.01(12)
C(10)-C(11)-C(12)	103.13(13)	C(5)-C(11)-C(12)	115.42(12)
C(13)-C(12)-C(11)	106.91(13)	C(14)-C(13)-C(12)	100.85(13)
C(14)-C(13)-C(16)	111.77(14)	C(12)-C(13)-C(16)	110.81(14)
O(5)-C(14)-C(13)	116.88(14)	O(5)-C(14)-C(10)	111.55(13)
C(13)-C(14)-C(10)	101.53(13)	C(16)-C(15)-C(9)	118.43(13)
O(6)-C(16)-C(15)	105.03(13)	O(6)-C(16)-C(13)	112.02(14)
C(15)-C(16)-C(13)	114.27(13)	N(1)-C(17)-C(1)	114.73(14)
N(1)-C(18)-C(5)	110.42(12)	N(1)-C(18)-C(8)	116.27(13)
C(5)-C(18)-C(8)	100.81(12)	N(1)-C(19)-C(20)	112.16(15)
O(1)-C(21)-C(1)	110.08(14)	Symmetry transformations used to generate equivalent atoms	

Table 3.4 Anisotropic displacement parameters ($Å^2$ x 10^3) for lycoctonine. The anisotropic displacement factor exponent takes the form: -2 gpi^2 [h^2 a^{*2} U11 + ... + 2 h k a* b* U

Atom	U11	U22	U33	U23	U13	U12
O(1)	32(1)	38(1)	52(1)	13(1)	11(1)	10(1)
O(2)	29(1)	36(1)	22(1)	-2(1)	-1(1)	10(1)
O(3)	29(1)	19(1)	32(1)	-3(1)	9(1)	-5(1)
O(4)	28(1)	25(1)	24(1)	-6(1)	2(1)	2(1)
O(5)	30(1)	34(1)	29(1)	-4(1)	12(1)	1(1)
O(6)	24(1)	37(1)	35(1)	-3(1)	0(1)	8(1)
O(7)	41(1)	33(1)	23(1)	-5(1)	-3(1)	8(1)
O(8)	37(1)	46(1)	40(1)	8(1)	0(1)	-14(1)
N(1)	34(1)	18(1)	25(1)	2(1)	14(1)	0(1)
C(1)	28(1)	20(1)	31(1)	2(1)	12(1)	2(1)
C(2)	37(1)	23(1)	33(1)	-2(1)	17(1)	2(1)
C(3)	42(1)	20(1)	25(1)	-3(1)	13(1)	1(1)
C(4)	31(1)	17(1)	21(1)	1(1)	5(1)	1(1)
C(5)	25(1)	18(1)	19(1)	1(1)	6(1)	0(1)
C(6)	24(1)	17(1)	26(1)	3(1)	6(1)	2(1)
C(7)	22(1)	24(1)	24(1)	-2(1)	3(1)	1(1)
C(8)	22(1)	18(1)	23(1)	-1(1)	5(1)	-2(1)
C(9)	23(1)	20(1)	21(1)	-3(1)	5(1)	0(1)
C(10)	24(1)	21(1)	20(1)	0(1)	6(1)	-1(1)
C(11)	25(1)	20(1)	22(1)	1(1)	7(1)	-3(1)
C(12)	25(1)	26(1)	32(1)	-6(1)	5(1)	-5(1)
C(13)	24(1)	28(1)	29(1)	-2(1)	7(1)	-4(1)
C(14)	28(1)	26(1)	27(1)	1(1)	10(1)	-1(1)
C(15)	25(1)	20(1)	31(1)	0(1)	7(1)	2(1)
C(16)	24(1)	29(1)	24(1)	-1(1)	4(1)	3(1)
C(17)	31(1)	24(1)	35(1)	2(1)	16(1)	-1(1)
C(18)	22(1)	18(1)	21(1)	1(1)	5(1)	-1(1)
C(19)	43(1)	20(1)	32(1)	4(1)	17(1)	-1(1)
C(20)	97(2)	36(1)	48(1)	13(1)	44(1)	10(1)
C(21)	27(1)	28(1)	42(1)	1(1)	13(1)	4(1)
C(22)	39(1)	48(1)	31(1)	-2(1)	-7(1)	16(1)
C(23)	37(1)	44(1)	42(1)	-4(1)	16(1)	-2(1)
C(24)	32(1)	55(1)	58(1)	0(1)	-12(1)	8(1)
C(25)	46(1)	49(1)	42(1)	-19(1)	-12(1)	0(1)

Table 3.5 Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for lycoctonine.

Atom	x	y	z	U(eq)
H(1)	6726	596	8599	48
H(3)	3315	7890	8324	31
H(4)	2745	6155	9688	32
H(2A)	4890	808	6357	35
H(2B)	4232	-58	7099	35
H(3A)	2809	74	5634	34
H(3B)	3027	2095	5571	34
H(4A)	1850	383	6833	28
H(6)	3626	1703	8367	26
H(7)	4624	4876	8425	28
H(10)	1852	3572	9253	26
H(11)	1249	1596	8110	26
H(12A)	-147	3446	6605	34
H(12B)	-613	1947	7186	34
H(13)	-1650	4138	7672	32
H(14)	-258	2919	8970	32
H(15A)	498	7850	8393	30
H(15B)	1000	7689	7469	30
H(16)	-563	6153	6733	31
H(17A)	4932	5121	7126	34
H(17B)	4825	3999	6200	34
H(18)	1516	5205	6651	25
H(19A)	3856	7255	6270	36
H(19B)	2400	7114	5839	36
H(20A)	3424	7472	4626	84
H(20B)	2761	5640	4523	84
H(20C)	4217	5778	4955	84
H(21A)	6422	2359	7489	38
H(21B)	6062	3439	8313	38
H(22A)	5135	2476	9768	63
H(22B)	4961	3531	10661	63
H(22C)	5663	4385	9946	63
H(23A)	-2021	5739	9046	60
H(23B)	-1469	5898	10154	60
H(23C)	-1663	4050	9669	60
H(24A)	-2452	7541	6230	78
H(24B)	-3206	8065	6987	78
H(24C)	-2929	6091	6828	78
H(25A)	741	-377	5147	74
H(25B)	-542	625	5001	74
H(25C)	25	-345	5969	74
H(8A)	1030(20)	5130(50)	10630(16)	79(10)
H(8B)	1430(30)	4120(30)	11480(20)	89(11)

Table 4.1 Crystal data and structure refinement for delpheline.

Identification code	k04farm3
Empirical formula	$C_{25}H_{39}NO_6$
Formula weight	449.57
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 11.9830(1)Å α = 90°
	b = 13.1780(1)Å β = 90°
	c = 14.3840(1)Å γ = 90°
Volume	2271.41(3) $Å^3$
Z	4
Density (calculated)	1.315 Mg/m^3
Absorption coefficient	0.093 mm^{-1}
F(000)	976
Crystal size	0.60 x 0.50 x 0.50 mm
Theta range for data collection	3.53 to 30.03°
Index ranges	-15<=h<=16; -16<=k<=18; -20<=l<=20
Reflections collected	56591
Independent reflections	6618 [R(int) = 0.0400]
Reflections observed (>2σ)	6240
Data Completeness	0.993
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6618 / 0 / 296
Goodness-of-fit on F^2	1.046
Final R indices [I>2σ(I)]	R^1 = 0.0372 wR_2 = 0.0981
R indices (all data)	R^1 = 0.0422 wR_2 = 0.1019
Absolute structure parameter	0.0(5)
Largest diff. peak and hole	0.747 and -0.310 $eÅ^{-3}$

Notes: H-bonded ribbons in lattice

Hydrogen bonds with H..A < r(A) + 2.000 Angstroms and <DHA > 110 deg.

D-H	d(D-H)	d(H..A)	<DHA	d(D..A)	A
O4-H4	0.840	2.096	158.70	2.894	O6 [-x+1, y-1/2, -z+3/2]

Table 4.2 Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for delpheline. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)
O(1)	9889(1)	9279(1)	6141(1)	28(1)
O(2)	6540(1)	8238(1)	8500(1)	20(1)
O(3)	5124(1)	9023(1)	7674(1)	20(1)
O(4)	5529(1)	7326(1)	6496(1)	21(1)
O(5)	4951(1)	10959(1)	6313(1)	22(1)
O(6)	6593(1)	12083(1)	7568(1)	26(1)
N(1)	8908(1)	7794(1)	7770(1)	21(1)
C(1)	9277(1)	8413(1)	5813(1)	21(1)
C(2)	10005(1)	7472(1)	5931(1)	24(1)
C(3)	9342(1)	6495(1)	5868(1)	25(1)
C(4)	8352(1)	6501(1)	6542(1)	20(1)
C(5)	7531(1)	7339(1)	6242(1)	17(1)
C(6)	8109(1)	8401(1)	6291(1)	17(1)
C(7)	7358(1)	9232(1)	5819(1)	18(1)
C(8)	6158(1)	9405(1)	6224(1)	17(1)
C(9)	6194(1)	9316(1)	7281(1)	17(1)
C(10)	6860(1)	8373(1)	7546(1)	16(1)
C(11)	6573(1)	7405(1)	6957(1)	18(1)
C(12)	7864(1)	10327(1)	5789(1)	24(1)
C(13)	6971(1)	11049(1)	6169(1)	22(1)
C(14)	5905(1)	10494(1)	5912(1)	20(1)
C(15)	7096(1)	11158(1)	7232(1)	20(1)
C(16)	6549(1)	10303(1)	7792(1)	20(1)
C(17)	8124(1)	8518(1)	7359(1)	17(1)
C(18)	8728(1)	6719(1)	7555(1)	22(1)
C(19)	7802(1)	5448(1)	6515(1)	28(1)
C(20)	9071(1)	7958(1)	8772(1)	29(1)
C(21)	10175(1)	7570(1)	9119(1)	35(1)
C(22)	10819(1)	9520(1)	5575(1)	34(1)
C(23)	5390(1)	8540(1)	8534(1)	21(1)
C(24)	3942(1)	10546(1)	5971(1)	27(1)
C(25)	7239(1)	12961(1)	7429(1)	40(1)

Table 4.3 Bond lengths [Å] and angles [°] for delpheline. Symmetry transformations used to generate equivalent atoms

O(1)-C(22)	1.4165(17)	O(1)-C(1)	1.4353(14)
O(2)-C(23)	1.4348(14)	O(2)-C(10)	1.4357(13)
O(3)-C(23)	1.4276(14)	O(3)-C(9)	1.4530(13)
O(4)-C(11)	1.4201(14)	O(5)-C(24)	1.4139(16)
O(5)-C(14)	1.4192(14)	O(6)-C(25)	1.4073(17)
O(6)-C(15)	1.4423(14)	N(1)-C(17)	1.4630(15)
N(1)-C(18)	1.4665(16)	N(1)-C(20)	1.4699(15)
C(1)-C(2)	1.5267(18)	C(1)-C(6)	1.5596(15)
C(2)-C(3)	1.5154(18)	C(3)-C(4)	1.5321(17)
C(4)-C(19)	1.5367(17)	C(4)-C(5)	1.5396(16)
C(4)-C(18)	1.5515(17)	C(5)-C(11)	1.5430(15)
C(5)-C(6)	1.5636(15)	C(6)-C(17)	1.5446(15)
C(6)-C(7)	1.5713(16)	C(7)-C(12)	1.5653(16)
C(7)-C(8)	1.5693(16)	C(8)-C(9)	1.5259(15)
C(8)-C(14)	1.5331(16)	C(9)-C(10)	1.5256(15)
C(9)-C(16)	1.5533(16)	C(10)-C(17)	1.5499(15)
C(10)-C(11)	1.5693(15)	C(12)-C(13)	1.5335(17)
C(13)-C(14)	1.5187(17)	C(13)-C(15)	1.5416(17)
C(15)-C(16)	1.5333(16)	C(20)-C(21)	1.5036(19)
C(22)-O(1)-C(1)	113.03(10)	C(23)-O(2)-C(10)	104.81(8)
C(23)-O(3)-C(9)	104.95(8)	C(24)-O(5)-C(14)	112.42(9)
C(25)-O(6)-C(15)	114.64(10)	C(17)-N(1)-C(18)	116.76(9)
C(17)-N(1)-C(20)	112.72(10)	C(18)-N(1)-C(20)	111.60(10)
O(1)-C(1)-C(2)	108.50(10)	O(1)-C(1)-C(6)	108.78(9)
C(2)-C(1)-C(6)	117.07(10)	C(3)-C(2)-C(1)	112.59(10)
C(2)-C(3)-C(4)	111.32(10)	C(3)-C(4)-C(19)	108.09(10)
C(3)-C(4)-C(5)	108.73(10)	C(19)-C(4)-C(5)	111.48(10)
C(3)-C(4)-C(18)	111.74(10)	C(19)-C(4)-C(18)	108.38(10)
C(5)-C(4)-C(18)	108.44(9)	C(4)-C(5)-C(11)	109.22(9)
C(4)-C(5)-C(6)	110.26(9)	C(11)-C(5)-C(6)	104.39(9)
C(17)-C(6)-C(1)	115.27(9)	C(17)-C(6)-C(5)	97.99(9)
C(1)-C(6)-C(5)	112.77(9)	C(17)-C(6)-C(7)	111.55(9)
C(1)-C(6)-C(7)	108.42(9)	C(5)-C(6)-C(7)	110.57(9)
C(12)-C(7)-C(8)	103.36(9)	C(12)-C(7)-C(6)	115.62(10)
C(8)-C(7)-C(6)	117.76(9)	C(9)-C(8)-C(14)	111.71(9)
C(9)-C(8)-C(7)	109.44(9)	C(14)-C(8)-C(7)	102.03(9)
O(3)-C(9)-C(10)	98.55(8)	O(3)-C(9)-C(8)	112.49(9)
C(10)-C(9)-C(8)	109.09(9)	O(3)-C(9)-C(16)	106.28(9)
C(10)-C(9)-C(16)	114.87(9)	C(8)-C(9)-C(16)	114.46(9)
O(2)-C(10)-C(9)	101.48(8)	O(2)-C(10)-C(17)	116.28(9)
C(9)-C(10)-C(17)	111.56(9)	O(2)-C(10)-C(11)	110.95(9)
C(9)-C(10)-C(11)	114.32(9)	C(17)-C(10)-C(11)	102.75(8)
O(4)-C(11)-C(5)	109.95(9)	O(4)-C(11)-C(10)	120.32(9)
C(5)-C(11)-C(10)	104.08(9)	C(13)-C(12)-C(7)	107.00(10)
C(14)-C(13)-C(12)	101.57(10)	C(14)-C(13)-C(15)	111.58(10)
C(12)-C(13)-C(15)	110.13(10)	O(5)-C(14)-C(13)	111.73(9)
O(5)-C(14)-C(8)	116.37(10)	C(13)-C(14)-C(8)	102.33(10)
O(6)-C(15)-C(16)	105.42(9)	O(6)-C(15)-C(13)	111.80(10)
C(16)-C(15)-C(13)	114.28(10)	C(15)-C(16)-C(9)	118.97(10)
N(1)-C(17)-C(6)	110.17(9)	N(1)-C(17)-C(10)	118.44(10)
C(6)-C(17)-C(10)	98.56(9)	N(1)-C(18)-C(4)	114.80(10)
N(1)-C(20)-C(21)	113.14(11)	O(3)-C(23)-O(2)	107.95(9)

Table 4.4 Anisotropic displacement parameters ($Å^2$ x 10^3) for delpheline. The anisotropic displacement factor exponent takes the form: -2 gpi^2 [h^2 a^{*2} U11 + ... + 2 h k a* b* U

Atom	U11	U22	U33	U23	U13	U12
O(1)	21(1)	24(1)	38(1)	-9(1)	6(1)	-5(1)
O(2)	22(1)	23(1)	15(1)	2(1)	3(1)	3(1)
O(3)	19(1)	23(1)	19(1)	4(1)	4(1)	1(1)
O(4)	19(1)	22(1)	24(1)	-2(1)	0(1)	-3(1)
O(5)	23(1)	20(1)	23(1)	-3(1)	-2(1)	4(1)
O(6)	23(1)	17(1)	38(1)	-7(1)	3(1)	0(1)
N(1)	23(1)	22(1)	18(1)	-4(1)	-3(1)	5(1)
C(1)	19(1)	22(1)	21(1)	-5(1)	3(1)	-2(1)
C(2)	20(1)	25(1)	27(1)	-6(1)	4(1)	1(1)
C(3)	24(1)	22(1)	27(1)	-7(1)	5(1)	3(1)
C(4)	22(1)	17(1)	22(1)	-3(1)	2(1)	2(1)
C(5)	20(1)	15(1)	17(1)	-3(1)	2(1)	0(1)
C(6)	18(1)	17(1)	15(1)	-3(1)	2(1)	-1(1)
C(7)	22(1)	17(1)	15(1)	-1(1)	3(1)	0(1)
C(8)	20(1)	17(1)	15(1)	0(1)	1(1)	1(1)
C(9)	18(1)	16(1)	16(1)	0(1)	3(1)	1(1)
C(10)	19(1)	17(1)	13(1)	0(1)	2(1)	1(1)
C(11)	19(1)	15(1)	19(1)	-1(1)	1(1)	0(1)
C(12)	27(1)	18(1)	27(1)	1(1)	10(1)	-2(1)
C(13)	26(1)	16(1)	23(1)	2(1)	3(1)	1(1)
C(14)	24(1)	18(1)	18(1)	0(1)	1(1)	2(1)
C(15)	20(1)	16(1)	24(1)	-2(1)	1(1)	2(1)
C(16)	26(1)	17(1)	18(1)	-3(1)	2(1)	1(1)
C(17)	19(1)	17(1)	16(1)	-2(1)	0(1)	1(1)
C(18)	25(1)	20(1)	22(1)	0(1)	0(1)	5(1)
C(19)	32(1)	17(1)	34(1)	-4(1)	4(1)	2(1)
C(20)	31(1)	36(1)	19(1)	-6(1)	-5(1)	10(1)
C(21)	32(1)	47(1)	26(1)	-2(1)	-9(1)	9(1)
C(22)	24(1)	30(1)	50(1)	0(1)	8(1)	-5(1)
C(23)	22(1)	24(1)	19(1)	2(1)	4(1)	1(1)
C(24)	25(1)	27(1)	31(1)	-7(1)	-2(1)	1(1)
C(25)	32(1)	27(1)	60(1)	-7(1)	-3(1)	-3(1)

Table 4.5 Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for delpheline.

Atom	x	y	z	U(eq)
H(4)	5015	7292	6893	26
H(1)	9151	8509	5131	25
H(2A)	10589	7470	5445	29
H(2B)	10382	7502	6543	29
H(3A)	9835	5913	6015	29
H(3B)	9064	6407	5225	29
H(5)	7235	7204	5604	21
H(7)	7257	9016	5158	22
H(8)	5611	8915	5951	21
H(11)	6642	6805	7378	21
H(12A)	8547	10361	6175	29
H(12B)	8062	10513	5143	29
H(13)	7011	11726	5856	26
H(14)	5823	10502	5220	24
H(15)	7909	11174	7385	24
H(16A)	5876	10586	8095	24
H(16B)	7073	10109	8293	24
H(17)	8349	9225	7527	21
H(18A)	8155	6448	7985	27
H(18B)	9430	6346	7675	27
H(19A)	7660	5255	5868	41
H(19B)	7095	5468	6857	41
H(19C)	8301	4950	6803	41
H(20A)	8464	7613	9116	35
H(20B)	9018	8693	8905	35
H(21A)	10237	7699	9788	52
H(21B)	10781	7920	8793	52
H(21C)	10228	6839	9002	52
H(22A)	11443	9069	5730	51
H(22B)	11039	10226	5683	51
H(22C)	10619	9431	4919	51
H(23A)	4907	7939	8628	26
H(23B)	5268	9016	9057	26
H(24A)	3974	10508	5291	41
H(24B)	3317	10981	6158	41
H(24C)	3836	9864	6228	41
H(25A)	7963	12881	7738	59
H(25B)	6849	13549	7690	59
H(25C)	7356	13065	6761	59

Appendix 2

Three Abstracts presented at international meetings:

BPC, Manchester, UK, 2004

BPC, Manchester, UK, 2005

The Sixth Princess Chulabhorn International Science Congress, Bangkok Thailand, 2007, -

The Interface of Chemistry and Biology in the Omics Era: Drug Discovery Natural Products

Extraction of Delphinium Pacific Giant Seeds

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Delphinium and *Consolida* species are highly toxic to cattle, of economic concern across North American ranges (Panter et al 2002). The norditerpenoid alkaloid methyllycaconitine (MLA) is the constituent which contributes a major part of this toxification and is a most potent, selective, and competitive antagonist for nicotinic acetylcholine receptors. The molecular basis for selective receptor blockade makes MLA and other related norditerpenoid alkaloids lead molecules in this research area (Hardick et al 1996). The basic nitrogen atom in MLA is a part of a substituted *N*-ethylpiperidine moiety. However, the corresponding *N*-ethyl nitrogen atoms in piperidine containing polycyclic norditerpenoid alkaloids are found to be significantly weaker bases than *N*-ethylpiperidine (pKa = 10.45). Thus, substitution with secondary alcohol and/or *O*-methyl ether functional groups acts to reduce the basicity, so that typically observed pKa values ("apparent" in 50% aq. ethanol, as they are only sparingly aqueous soluble) include: elatine (5.33), condelphine (6.45), neoline (6.70), aconitine (7.23), lycoctonine (7.50) (Golkiewicz et al 1968), and delpheline (7.6). The basicity of inuline (anthranoyl lycoctonine) and MLA are not yet reported, but this physico-chemical parameter will significantly modulate the fractions in which MLA is discovered during the isolation protocol and its distribution in biological systems. Ground Delphinium Pacific Giant seeds (500 g) were extracted in a soxhlet thimble with hexane, dichloromethane, and ethanol sequentially (5 cycles each, 2 L scale). After concentration in vacuo the residues from each extract were extracted with 0.5 M H_2SO_4 (4 x 200 mL). The pH of the combined acidic layers was adjusted to 4.6 with solid $NaHCO_3$, to pH 7.1 with solid Na_2CO_3, and to pH 10.0 with 0.1 M NaOH; each fraction was back extracted with dichloromethane (5 x 100 mL). The combined organic extracts were washed with water (1 x 50 mL), dried ($MgSO_4$), and concentrated in vacuo yielding 3 fractions (F) 1, 2, and 3 respectively, total alkaloid yields were: F1 (1.5 g), F2 (1.5 g), and F3 (0.5 g) from the hexane extract; F1 (3.5 g), F2 (2.1 g), and F3 (0.6 g) from the dichloromethane extract; F1 (2.4 g), F2 (0.8 g), and F3 (0.4 g) from the ethanol extract. TLC analysis (cyclohexane-chloroform-diethylamine 5:4:1) showed that MLA was present in each fraction of three solvent extracts except F3 of the ethanol extract. Delpheline was present in F1 and F2 of all three solvent extracts. MLA was isolated by column chromatography and its structure established on the basis of ^1H, ^{13}C, DEPT, COSY, HMQC, and HMBC NMR spectroscopic techniques. We are now purifying MLA to homogeneity to determine its "apparent" pKa value.

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Golkiewicz, W. et al (1968) *Diss. Pharmac. Pharmacol.* **20:** 635-639
Panter, K. E. et al (2002) *Biochem Syst. Ecol.* **30:** 113-128

Structures of Norditerpenoid Alkaloids from Delphinium Pacific Giant Seeds

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Plants in the genera *Delphinium* and *Aconitum* are a major source of norditerpenoid alkaloids. Some of these alkaloids are highly toxic to mammalian species, and therefore their mode(s) of action are of interest to biological and medicinal chemists and pharmacologists. Pelletier & Joshi (1987) summarized the early X-ray crystallographic studies of norditerpenoid alkaloids. These norditerpenoid alkaloids have been categorized into three broad groups: the aconitine-type which lacks of an oxygen function at C-7, the lycoctonine-type characterized by bearing an oxygen function at C-7, and finally three synthetic compounds where X-ray data have been published (Pelletier & Joshi 1987). These natural products, and their derivatives and analogues, are ligands with important biological activity at selected ion channels. The biological activity and aspects of the history and ethnopharmacy of Delphinium usage have been elegantly covered by Benn & Jacyno (1983). Ground Delphinium Pacific Giant seeds (500 g) were extracted in a soxhlet thimble sequentially with: hexane, dichloromethane, and ethanol (5 cycles each, 2 L scale) (Goodson 1943). After concentration in vacuo, the residues from each extract were extracted with 0.5 M sulfuric acid (4 x 100 mL). The combined acidic layers was basified with 5M NaOH; each fraction was back-extracted with dichloromethane (4 x 150 mL). The combined organic extracts were washed with water (1 x 50 mL), dried ($MgSO_4$), and concentrated in vacuo, total alkaloid yields were: the hexane extract (4.0 g); the dichloromethane extract (1.8 g); the ethanol extract (5.9 g). TLC analysis (cyclohexane-chloroform-diethylamine 5:4:1) showed that MLA and delpheline was present in all three solvent extracts (Goodson 1943). MLA and delpheline were isolated by column chromatography and their structures established on the basis of ^{1}H, ^{13}C, DEPT, COSY, HMQC and HMBC NMR spectroscopic techniques. Furthermore, delpheline was recrystallized from ethanol:hexane (1:1) and studied for the first time by X-ray crystallography. As a result, the conformations of the six rings are: A and E, chair; D, half-chair (boat flattened at C-15); C and F, envelopes; and B, boat. Three other known alkaloids, namely aconitine, mesaconitine, and lycoctonine, were also studied by X-ray crystallography for comparison. All showed similar conformations, with only slight differences in the shape of ring D. It is interesting to note that these similar alkaloids differ considerably in their biological activities that seem to depend more upon the patterns and types of substitution than on the conformations of the norditerpenoid alkaloid framework.

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DELPHELINE, PACININE, AND DELAVAINE - NORDITERPENOID ALKALOIDS FROM DELPHINIUM PACIFIC GIANT SEEDS

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Plants in the genera *Delphinium*, *Consolida*, and *Aconitum* are a major source of norditerpenoid alkaloids that are highly toxic to mammals. However, *Delphinium* extracts have been used as medicines for many centuries in different cultures. There have been many claims to their efficacy for a wide variety of ailments. Therefore, their modes of action are of interest to pharmacologists and to biological and medicinal chemists as these natural products, their derivatives and analogues, are ligands with important biological activities at selected ion channels e.g. methyllycaconitine (MLA) is a selective competitive antagonist at α7 sub-type nicotinic acetylcholine receptors (nAChR); α7-selective agonists also have potential as therapeutics in modern drug discovery.

Delpheline, its B-ring C6-ketone pacinine (oxidized C6-β-OH delpheline), and the methyl esters delavaines A and B (a 3:2 mixture, possibly artefacts from opening the MLA succinimide ring with methanol during isolation, cf delsemines A and B, amides from ring opening with ammonia) were isolated from ground Delphinium Pacific Giant seeds and their structures established by NMR spectroscopy and high resolution mass spectrometry. Their biological activities were determined in competitive α-bungarotoxin binding assays for α7 nAChR in rat brain membranes. Delavaines A and B were potent ligands (IC_{50} = 50 nM, cf MLA IC_{50} = ~1-2 nM), whereas pacinine and delpheline displayed only modest activity at α7 nAChR (IC_{50} = ~1 μM). These results allow us to begin to map the determinants of activity at α7 nAChR.

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